STUDIES ON THE WILT DISEASE OF GUAVA

(*Psidium guajava* L.)

DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

Master of Philosophy

IN

AGRICULTURE
(PLANT PROTECTION)

BY

ASMA NAZ

DEPARTMENT OF PLANT PROTECTION
FACULTY OF AGRICULTURAL SCIENCES
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2009

DEDICATION


HOLY
PROPHET
(SAW)


MY
ADOARBLE
PARENTS
&
SIBLINGS


MY RESPECTED
SUPERVISOR

Department of Plant Protection

Dr. SHABBIR ASHRAF
Reader

Faculty of Agricultural Sciences
Aligarh Muslim University,
Aligarh- 202 002 (India)
Phone: (0571) -2901524, +3765
(0571)-2702208
e-mail: shabbiragri@yahoo.com

August 18, 2009

CERTIFICATE

This is to certify that **Ms. Asma Naz** has worked under my guidance on the problem, **Studies on wilt disease of Guava (*Psidium guajava* L.)** in partial fulfillment of the requirement for the award of degree of **M. Phil (Agriculture) Plant Protection**. The work done by her is original and up-to-date. She is allowed to submit the dissertation for evaluation.

28/8/09

(SHABBIR ASHRAF)

Acknowledgements

*In the name of **ALMIGHTY ALLAH**, who is the source of entire knowledge and wisdom endowed to mankind and **His HOLY PROPHET MOHAMMAD** (Peace Be Upon Him), who is for ever a torch of guidance and knowledge for humanity as a whole.*

I feel highly privileged to express my heartiest gratitude to my supervisor Dr. Shabbir Ashraf, Reader Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, for his guidance, keen interest, best cooperation and constructive criticism throughout my study. His kindness and generous response to my difficulties during the research work never forgotten.

I gratefully acknowledge Professor Akhter Haseeb, Chairman Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh for constant support, encouragement and providing necessary facilities.

My sincere thanks to Professor P. Q. Rizvi, Dean Faculty of Agricultural Sciences, Aligarh Muslim University Aligarh for corporation and support.

My heartfelt thanks to my teachers, Dr. M.R. Khan, Dr. M.S. Ansari, Dr. R.U. Khan, Dr. M. Haseeb, for their help and support.

I would like to give special thanks to my senior and my sister Huma Naz for her help and cooperation specially during my project work.

I am in debted to, Ritu Aapa, Arshad Anwar Bhai, Haider Bhai, for his precious and constructive suggestion and help in order to make this work success. I am also thankful to Darakshan Aapa, Bushra Aapa, Kamran Nadeem bhai, Fazil Hasan, Fiyaz Bhai, Reshu aapa, Wajid Ali Bhai,

Sahar, Shakeel for their help and support during my project. I would also like to extend my thanks to Aman Bhai, Raghib Bhai, Iftetah Bhai and Akbar Bhai, Amir Bhai, Nahid mam, Shariq Bhai for their cooperation.

I wish to acknowledge my classmates Vishv Deepak Sharma and Rafat Aapa, my friends, Sadaf rizwan, Shaheen, Saba Ajaz, Anamika and Saba Iqbal for creating an ideal milieu to strengthen my thoughts and gain knowledge.

I would also like to present a bouquet of thanks to my Father Mr. Maqsood Ahmad Siddiquie, Mother Mrs. Shahana Parveen, for their unconditional love and never ending care and support. They left no stone unturned in fulfilling my each and every wish and needs.

I feel pleasure in expressing my hearty thanks to my Brother Zaeem, Suleman, Sister Ayesha, Samiya and my Abba, Bari Ammi who shared all my joys and troubles and whose encouragement was a source of inspiration and strength through the thick and thin in accomplishing this task. I also like to thank my Grannies for their enormous affection, care and moral support.

AsmaNaz

ASMA NAZ

Contents

LIST OF TABLES

TABLES

LIST OF PLATES

PLATES

Chapter-1
Introduction

INTRODUCTION

Agriculture forms the basis of economy of almost all the countries. India occupies outstanding figures in the world having several agricultural products and stands in a unique position to grow almost every possible crop. Agricultural produce in India comprises a variety of cereals, pulses, oilseeds, beverages, fruits, vegetables, plant fibers and other industrial raw-materials.

In India, where larger part of agriculture is rainfed growers of seasonal crops like cereals, pulses, oilseeds, vegetables etc. constantly apprehend crop failure due to scarcity of excess of rainfall. Fruits specially the perennial ones, are nearly free from this danger unless the natural calamity is a prolonged one. Thus growing of such fruits is an insurance against such natural hydrological hazards.

India is the second largest producer of fruits in the world. Fruits grown in area of over 3.73 million ha in India, contributing to an annual production of 46.04 million tonnes (2001). Indian fruit basket comprises a wide variety ranging form the tropical fruits like mango, banana, citrus, papaya, guava, sapota and grape, to apple, pear, peach, and walnut among the temperate fruits and aonla, ber, pomegranate, anona, fig, phalsa etc. among the arid zone fruits.

Fruits provide protective and wholesome food for maintenance of human health. They are rich in carbohydrate, minerals and vitamins. Fruits are necessary for making balanced and healthy diets.

Fruits provide good source of energy with a unique capacity to guard against many deficiency diseases. Being anti-oxidant, they cause delay in progress of senility in human body.

Fruits provide vitamins, the deficiency of which causes ailments known as deficiency diseases. An illustration is provided below:-

Name of the vitamin	Source of availability	Deficiency diseases
Vitamin A	Mango, papaya, persimmon, jackfruit, date, walnut etc.	Night blindness, xeropthalmi, phrynderma.
Vitamin B	Almond, apricot, banana, cashewnut, walnut etc.	Beri-beri, paralysis.
Vitamin B-12(Riboflavin)	Bael, papaya, pineapple, pomegranate, litchi, cashew	Loss of weight, lack of appetite, sore throat, cateract.
Vitamin C	Aonla, cherry, citrus, bael, mango, **guava**, ber, papaya.	Scurvy.

Guava (*Psidium guajava*) is one of the important fruit crops of sub tropical countries. It occupies 151.5 thousands ha and yields about 16.314 lack tonnes fruits per year in India (Anonymous 1999). In India it is grown almost in all states. Mostly it is grown in Bihar, Uttar predesh, Madhya Pradesh, Maharashtra, Karnataka, Punjab, Andhra Pradesh and Uttranchal. Bihar is the leading state in guava production with 2.959 lack tones of fruit per year and occupies

24.463 thousand ha (Anonymous 1999). In U.P, Allahabad is recognized for best quality of guava in India as well as in world.

Guava has potential in fruit industry of our country because of its delicious taste, aroma, sweetness, flavour and balance amount of acid, sugar and pectin (Singh and Rao 1996). Guava varieties bear three crops in a year viz. rainy, winter and summer. Which contribute to 70, 27 and 3 percent yields respectively (Singh and Reddy 1997).

It is a hardy crop and is cultivated successfully even in neglected soils and is attacked by a large number of pathogens, mainly fungi. About 177 pathogens (167 fungi, 3 bacteria, 3 algae, 3 nematodes and epiphyte) are reported on various parts of the crop including fruit causing various diseases. Wilt is the most destructive disease of guava in India and loss due to this disease substantial. As the disease is of soil borne in nature, there are limitations for the control of this disease.

Wilt of guava was first reported in 1935 from Allahabad. The wilt disease was found to be more prevalent during the rainy season. Infected plants develop chlorosis; leaf abscission followed by wilting of the entire plant (Das gupta and Rai 1947) reported that disease produces vascular wilt symptoms in guava (Singh and lal 1953) estimated that 5-51% of trees die due to guava wilt every year in twelve districts of U.P resulting loss of rupees one million approximately.

The exact cause of the diseases is not fully understood but the pathogen viz. *Fusarium oxysporum* f. sp. *psidii*, *F. solani*, *Acremonium diospyri* and *Gliocladium rosium* are some of the pathogens reported from various workers and may be the cause of guava wilt incitant.

The fungi comprise of more than 100,000 species having representation in almost every available habitat on earth and many fungi are of major economical and medicinal significance to man.

The fungi are small, generally microscopic, eukaryotic usually filamentous, branched, spore-bearing organism that lack chlorophyll and have cell walls that contain chitin, cellulose or both. Most known species are strictly saprophytic, living on dead organic matter, which they help to decompose. More than 8000 species of fungi can cause diseases in plants. All plants are attacked by some kinds of fungi and each of the parasitic fungi can attack one or more kind of plants.

At Aligarh conditions, *Fusarium oxysporum* f. sp. *psidii* was invariability found to be associated with guava wilt, which is one of the most important genera of the family Tuberculariaceae and belongs to order Moniliales under sub division Deuteromycotina.

In the effort to manage this disease, it is suggested that biological control agents should be used to control the wilt. However, such disease control measures require knowledge of various aspects of causal organism and disease development.

Therefore, keeping the above facts in mind and considering the serious nature of disease, the present investigation was under taken with following objectives:-

1. Survey.
2. Isolation of the pathogen.
3. Pathogenecity test of the fungus.
4. Measurment of the conidia.
5. Effect of temperature of the disease incidence.
6. Management the disease with biocontrol agents in vitro and in vivo.
7. Management of the disease with plant extract in vitro.

Chapter-2
Review of literature

REVIEW OF LITERATURE

Guava is one of the important fruit crop of India. It ranks fourth in production among fruit crops. Guava has good potential in fruit industry in our country because of its delicious taste, aroma, sweetness, flavour and balance amount of acid, sugar and pectin (Singh and Rao, 1996).

Several biotic and abiotic factors are responsible for low yield. Several microorganisms' viz. fungi, bacteria, viruses and nematodes cause diseases in guava Causal pathogens are both seed and soil borne. The important diseases of guava are wilt, anthracnose and fruit rot, grey blight, leaf spots and scab and zinc deficiency (Singh, 2000).

Geographical distribution

In India wilt of guava was reported for the first time reported in 1935 from Bakkarpur area of Allahabad. Das gupta and Rai. (1947) recorded the disease in a severe form in orchards of Lucknow while Dey (1948), reported it from Allahabad as well as from Kanpur and Lucknow. During 1949-50 guava trees suffered serious losses in 11 districts of U.P. (Anonymous, 1949). Prasad *et al.* (1952), estimated that guava wilt spreads rapidly to cover about 20,000 m^2 area in U.P. while Mathur (1956), reported that 15-30 per cent of trees in Allahabad, Farrukhabad and Unnao districts, 5-15 per cent in Kanpur and Jaunpur and less than 5% in Gorakhpur, Ballia, Hardoi, Barabanki and Varansi districts were affected by the disease. Edward


INDIA
J&K
HP
Punjab
Uttaranchal
Delhi
U.P.
Rajasthan
Bihar
Sikkim
Arunachal Pradesh
Nagaland
Assam
Manipur
Meghalaya
Tripura
Mizoram
Gujarat
M.P.
Jharkahand
W.B.
Orissa
Maharashtra
A.P.
Karnataka
Kerala
Tamilnadu
Lakshadweep
Andaman and
Nicobar
(Island)
Wilt Affected Area

and Shrivastva (1957), reported wilt as a most serious disease, which is threatening guava cultivation in U.P. The wilt disease was also reported from western districts of U.P. Singh and Lal (1953), and from union territory of Delhi (Anonymous, 1953). The disease appear in severe form in Varansi districts (Pandey and Dwivedi), 1985 and (Mishra Prakash, 1986). Mehta (1987) reported its occurrence in Kaimganj, areas of western U.P., Bithoor (Kanpur) Ganga Ghat (Unnao), Babakkarpur (Allahabad), Lucknow, Bichpuri (Agra) and Sasni (Aligarh). Occurrence of serious wilt was reported from guava orchards in West Bengal (Chattopadhyay and Sengupta, 1955), where it was confined mainly to the gangetic alluvium of Baruipur area in the district of 24 Parganas and in the lateritic zone of Jhargram, Midanpur and Kashkul in the district of Bankura (Chatopadhyay and Bhattacharya, 1968). The disease has also been reported to occur in Haryana (Suhag, 1976 and Mehta, 1987). Punjab (Chandra Mohan *et al.*, 1985), Rajasthan (Katyal, 1972; Bhargava *et al.*, 2003), and Andhra Pradesh (Jhooty *et al.*, 1984). Jharkhand (Srivastava *et al.*, 2001), Orissa (Das *et al.*, 1993). Recently the disease was recorded in severe form, from Indore, M.P. Wilt disease of guava has also been reported from Taiwan (Hsieh *et al.*, 1976; Leu *et al.*, 1979), South Africa (Grech, 1985, Vos *et al.*, 2000), Brazil (Tokeshi *et al.*, 1980 Rodrigues *et al.*, 1987 Junqueira *et al.*, 2001), Bangladesh (Hamiduzzaman *et al.*, 1997) and Canberra in Australia (Lim and Manicom, 2003).

Losses

Singh and Lal (1953), estimated 5-15% losses every year in 12 districts of U.P. which was around Rs. one million during 1953. In West Bengal, it reduced the yield about 18.16-22.79 q/ha in wilt-affected orchards. (Chatopadhyay and Sengupta, 1955). New seedlings and grafted plants, in affected areas showed stunted growth, which flowered rarely and succumbed to wilt within a very short time (Chatopadhyay and Bhattacharya, 1968). In Andhra Pradesh, 7,000 acres of land was under guava cultivation and the wilt disease had reduced the land value (Jhooty *et al.*, 1984). About 150 acres of guava orchards in Punjab and 300 acres in Haryana have been uprooted during 1978-81 (Jhooty *et al.*, 1984). In general, losses due to wilt in guava around Lucknow area vary from 5-60 % (Misra and Shukla, 2002).

Symptomatology

The affected plants parts show yellow coloration with slight curling of the leaves of the terminal branches. Plant at a later stage, show unthruftiness with yellow to reddish discoloration of leaves. Subsequently, there is premature shedding of leaves and fruit size becomes smaller. Some of the twigs become bare and fail to bring-forth new leaves or flowers and eventually dry up. Fruits of all the affected branches remain undeveloped, hard, stony and remain hanging in the tree. Later on, the entire plant becomes defoliated and eventually dies. It requires almost sixteen days for complete wilting.

Plate 1. Guava trees showing wilt symptoms, A: Dropping of leaves B: Yellowing of leaves C: Complete drying of a tree due to infection

Some affected trees linger on even up to 252 days and then die (Misra and Pandey, 2000b). Misra and Pandey, (2000b) also studied variation in the symptoms during different time of the year. They noticed yellowing of the leaves with the interveinal chlorosis during the month of August, which drop even with the slight shaking of the plants. During September, general drooping of the leaves takes place. During October, complete wilting of plants are seen with almost dried leaves and small dried black fruits hanging on the branch. A few plants also show partial wilting, during different months but later escape/resist wilting (Misra and Pandey, 2000b).

Chattopadhyay and Bhattacharya (1968), have reported that the roots also show rooting at the basal region. The finer roots show black streaks, which become prominent on removing the bark (Das Gupta and Rai, 1947). The root also shows rooting at the basal region and the bark is easily detachable from the cortex. The cortical regions of the stem and root show distinct discolouration and damage, light discolouration is also noticed in vascular tissues (Chattopadhyay and Bhattacharya, 1968). According to Singh and Lal (1953) and Edward (1960), the pathogens attack young as well as old fruit bearing trees (Misra and Shukla, 2002). New seedlings and grafts also show disease symptoms. The disease symptoms are identified viz; (a) slow and (b) sudden wilt/quick wilt caused by *Macrophomina phaseolina* which dominated in alkaline soil, was

related with slow wilt, while *Fusarium oxysporum* f. sp. *psidii* dominating in acidic soil was related with sudden wilt.

Causal pathogen

Large number of the pathogens has been isolated from the wilted plants by several workers in different parts of the world. The exact cause of the disease is still not fully understood, but the pathogens, viz. *Fusarium oxysporum* f. sp. *psidii*, *F. solani*, *Macrophomina phaeseolina*, *Rhizoctonia bataticola*, *Cephalosporium sp. Acremonium diospyri* and *Gliocladium roseum* were reported to be responsible for the guava wilt.

Before 1941, wilt was considered to be caused by *Cephalosporium sp.* in north India (Vestal, 1941). Dey (1948), invariably isolated *Cephalosporium* from the roots of wilted plants. Das Gupta and Rai (1947) reported the association of *Fusarium sp.* with the wilt disease in U.P. Later Prasad *et al.* (1952), attributed the wilt to *Fusarium oxysporum* and proposed the name *Fusarium oxsporum* f. sp. *psidii*. This view was supported by Edward and Shrivastva (1957) and Pandey and Dwivedi (1985). Edward (1960) also observed that the *F. oxysporum* f. sp. *psidii* exists in a variety of forms, which differ in their cultural and morphological characters. He reported that *F. oxysporum* f. sp. *psidii* penetrates either directly through the root piliferous layer of guava seedling through the opening caused by secondary roots. Hyphae are found in xylem vessels of inoculated plants.

Besides, the involvement of above pathogens, association of other pathogens with guava wilt also has been reported by different workers from different places. Tandon and Agarwal (1954) reported wilt and dieback of guava to be caused by *Gloeosporium psidii* from Allahabad. In West Bengal, *Macrophomina phaseolina*, and *Fusarium solani* (Mart) sacc. were found to cause wilt in guava either individually or in combination (Chatopadhyay and Sen Gupta, 1955). *M. phaseolina* was found to be more predominant in the indo-gangetic alluvial tracts and confined exclusively to the root region, while *F. solani* was found to be more prominent in the dry lateritic area of the West Bengal (Chatopadhyay and Bhattacharya, 1968). Both *M. phaseolina* and *F. solani* were found to incite the wilt disease either individually or in combination. In either case, the fungus first colonizes the surface of roots and then enters into its epidermal cells. There after, intercellular mycelium establishes first in the epidermal cell and then spreads into the cortical cells, which get considerably damaged and filled up with the mycelium. *F. solani* enters the xylem vessels, grows inside and blocks them. *M. phaseolina* first invades the phloem and destroys it. The xylem vessels, are also attacked in few cases (Chatopadhyay and Bhattacharya, 1968) and (Chatopadhyay and Sengupta, 1955). Histopathological observation made by various workers in naturally wilted and artificially inoculated plants revealed the presence of *F. solani*, *F. oxysporum* and *M. phaseolina* in vascular tissues

(Chattopadhyay and Bhattacharya, 1968a,b Edward, 1960c; Chandra Mohan, 1985; Pandey and Dwivedi, 1985; Sohi, 1983a,b). *Gliocladium vermoesenii carda,* a known saprophytic fungus, was found to be associated with the wilted plants in Punjab (Chandra Mohan, 1985).

In recent studies, at the Central Institute for Subtropical Horticulture, Lucknow out of several pathogens isolated from the wilt-affected guava plants, *Gliocladium roseum* was found to be the most potent pathogen, which could reproduce the symptoms of wilt in fields on a large scale in 4 to 8 years old trees, after artificial inoculation. The stem hole inoculation technique was standardized, which could reproduce the disease after two months of inoculation (Misra and Pandey, 1997). Pandit and Smajpati, (2002) reported wilt to be caused by *Botyodiplodia theobromae* in Midnapur (W.B.), while Gupta *et al.* (2003) reported association of *Verticillium albo-atrum* with guava wilt from Allahabad. Misra *et al.* (2004a) recorded wilting due to *Gliocladium roseum* in association with *Sclerotium rolfsii* and *Rhizoctonia bataticola.* Khan *et al.* (2001) indicated the role of nematodes as co- factor in guava wilt. They found *Helicotylenchous dihystera* in terms of population frequency and density to be the dominant species in wilted guava plants.

Various reports from other parts of the world are different. Webber (1928) reported *Clitocybe tabescens* killing guava tree in Florida (USA) in 1928. In Cuba three nematodes viz., *Meloidogyne*

sp., *Helicotylenchus* sp. and *Pratylenchus* sp. have been found associated (Rodriguez and Landa, 1977) with guava wilt. In Taiwan, the disease is reported to be caused by *Myxosporium psidii* Corda (Hsich *et al.*, 1976 and Leu and Kao, 1979). In South Africa, *Septofusidium* sp. was found to be associated with rapid wilt of guava plants (Grech, 1985).

In another report, *Acremonium diospyii* was given as the causal organism of the wilt (Joubert and Frean, 1993). From Brazil, *Pseudomonas* sp. (Tokeshi *et al.*, 1980) and *Erwinia psidii* (Rodgrigues *et al.*, 1987) and Planaltina (Jungueira *et al.*, 2001). *F. oxysporum* and *Colletotrichum gloeosporioides* (perfect stage Glomerella *cingulata*) are found to be associated with the disease and they are supposed to act synergistically, when they are present together (Ansar *et al.*, 1994), Hamiduzzaman *et al.*, (1997) reported from Bangladesh that wilt incidence was maximum when seedlings were inoculated by *F. oxysporum* f. sp. *psidii* along with nematodes *H. dehystera* and *H. indicus*. From South Africa, Vos *et al.* (2000) reported guava wilt disease caused by *Penicillium vermoesenii*.

For the first time the association of *Fusarium* sp. was reported by Dasgupta and Rai (1947), Later Prasad *et al.* (1952), attributed the wilt to *Fusarium oxysporum* and proposed the name *Fusarium oxsporum* f. sp. *psidii*. This view was supported by Edward and Shrivastva (1957) and Pandey and Dwivedi (1985). From Varansi *F. oxysporum* f. sp. *psidii*, *F. solani*, *F. coerudium*, *F. moniliforme* and

R. solani were also found on rhizoplane and rhizosphere of guava (Dwivedi, 1991a; Dwivedi and Dwivedi, 1999).

The fungus produces micro conidia which are oval to ellipsoidal, 1-2 celled, hyline and measure 5.8-9.5 x 3.5-4.5 µm The macro conidia are elongate curved at both ends, thin, hyaline and 3 or more septate. They measure 25.2-42 × 2.8-4.2 µm. The spherical chlamydospores, formed by hyphal and conidial cells, are terminal or intercalary, thick-walled and 10-15.5 µm in diameter. Several physiological variants of the fungus have been reported by Edward, J.C. (1960a), Singh, (1982).

In an early report Parsad, Mehta and Lal stated that Sporodochia and pionnotes are present in the culture. They further grouped and measured the conidia on the basis of their septation: 0 Septate - 7 – 10 x 2-3 microns; 1Septate - 15 –20 x 3-4 microns; 2 – Septate - 32 –40 x 3-6 microns; 3 – Septate - 37 –40 x 4-7 microns. Edward (1960b, c) explained that *F. oxysporum* f. sp. *psidii* exists in a variety of forms, which differ in their cultural and morphological characters. He reported that *F. oxysporum* f. sp. *psidii* penetrates either directly through the root piliferous layer of guava seedling through the opening caused by secondary roots. Hyphae are found in xylem vessels of inoculated plants. Lim and Manicom (2003) have also reported from Australia that reported wilt of guava is caused by *F. oxysporum* f. sp. *psidii*.

Epidemiology

Mehta (1951) reported severe incidence of wilt in alkaline soils at pH ranging from 7.5 to 9.0, while Sen and Verma (1954) reported high disease incidence in lateritic soil at pH 6.5. A soil saturation of 60-80% has been reported optimum for disease development in West Bengal (Chatopadhyay and Bhattacharya, 1968b). A pH 6.0 has been reported optimum for the development of the disease. Both pH 4.0 and 8.0 reduces the disease. Low incidence of the disease has been reported at Nitrogen 630 ppm N and is more both at higher as well as at lower levels of nitrogen. Moderate to high concentrations of phosphate (207-345 ppm) are effective in reducing the disease (Chattopadhyay and Bhattacharya, 1968b). Mehta (1987) reported more disease in clay loam and sandy loam compared to heavy soil types.

Guava seedlings are more susceptible to *Macrophomina phaseolina* as well as *F. solani* than the older plants of 3 years age. On the other hand Chattopadhyay and Bhattacharya (1967) reported that *F. solani* could infect guava plants from one month old plants to more than 4 years old trees. According to Misra and Shukla, (2002) guava plants above five-year old were more susceptible to the disease.

Infected guava plants start showing sign of the of wilting with the onset of rainy season in August with maximum number dying in September and October (Das Gupta and Rai, 1947; Edward, 1960a; Suhag, 1976).

Dwivedi *et al.* (1990) at Varanasi also found more pathogenic fungi during rainy season. The fungi survived better in association with root bits in adverse climatic conditions in the summer months, while in rainy and winter months they survive on roots. Extensive studies on the progress of natural wilting of guava plants during different months have been made by Misra and Pandey (1999a, d, 2000b) at Lucknow. They found maximum wilting during October. Some plants, which show slight yellowing, started recovering from December onwards. On analyzing the weather data, they found higher rainfall during July-September with maximum temperature ranging from 31.3 to 33.5^{0}C, minimum temperature ranging from 23 to 25^{0}C and humidity of 76 percent. They also found that minimum two months are required for the complete wilting of plants.

Disease management through chemicals

As early as 1949, control of wilt was achieved with Chaubatia paste (Anonymous, 1949) but this control measure is not considered valid, as guava wilt is a soil borne disease. Jain (1956) found chemotherapeuratic action of 0.1 per cent water-soluble 8 Quinolinol sulphate against the wilt pathogen (*Fusarium oxysporum* f. sp. *psidii*) It's injection in apparently healthy guava plants in a diseased area provided protection against wilt at least for one year and when injected into slightly wilted plants, it was beneficial for their partial recovery. Suhag (1976) reported control of wilt by severe pruning and then drenching with 0.2 per cent either. Benlate or Bavistin 4

times in a year and spraying twice with Metasystox and Zinc sulphate. But due to soil borne nature of the disease, pruning does not seem to control wilt. Misra and Pandey (1999b) reported that though different fungicides viz. Bavistin ,Topsin M, Indofil M-45, Thiram, Blitox check the various wilt pathogens in laboratory effectively. Moreover, these pathogens increase it's aggressiveness with profuse spore mass production in the soil, once the effect of these fungicides diminishes. Bhargava *et al.* (2003) also found control with thiophanate methyl in lab. In Taiwan, Carbendazim, Captafol and Thiabendazole proved effective against wilt pathogen under laboratory experiments but failed *in vivo* (Leu *et al.*, 1979). In South Africa tebuconazole, propiconazole, prochoraz, triforine and carbendazim + flusilazole were effective under *in vitro* evaluation (Joubert and Frean, 1993). Antibiotic action (Dwivedi 1990) and heavy metals viz. Hg, Cd and Cu were found effective for control of wilt (Dwivedi 1991b). Nematodes are reported to aggravate the wilt incidence in guava. Disinfection of soil with DBCP at 52.8ml/10m^2 or Metham sodium at 252.5ml/10m^2 was achieved to control nematodes (Rodriguez and Landa, 1977).

Besides fungicides some soil amendment chemicals cakes and fertilizers were also evaluated for control of wilt. Mathur *et al.* (1964) found control of wilt disease by soil treatment with 1.82 kg. Lime or gypsum/tree, although the control mechanism was not well understood. At. CISH, Lucknow wilt was controlled by application of

6 kg. Neem cake + 2 kg. Gypsum per plant (Misra and Pandey, 1994-95). Oil cakes like neem cake, mahua cake, kusum cake supplemented with urea @ 10 kg and 1 kg respectively also check the disease (Das Gupta and Ghoshal, 1977). Suhag and Khera (1986) advocated that spread of wilt could be checked by judicious amendments with nitrogen and zinc.

Disease management through cultural practices

Mathur (1956) advocated that wilt could be controlled by proper sanitation in the orchard. Wilted trees should be uprooted, burnt and trench should be dug around the tree trunk. Symptoms of the disease do not appear under green manuring and the disease development is less when organic sources of nitrogen are used (Chattopadhyay and Bhattacharya 1968b). Soil solarizations with 30mm transparent polyethylene sheet during May-June (Dwivedi, 1993b) have been suggested for the control of wilt pathogens. Prasad *et al.* (2003), Khan and Misra (2003) and Misra *et al.* (2004b) reported intercropping with turmeric or marigold to check the wilting of guava. These cultural practices are useful and may be adopted to escape wilt.

Disease management through varietal resistance

Edward (1961) suggested guava species *Psidium cattleianum* var. *lucidum* and *Syzigium cuminii* (Jamun), which seldom get attacked with wilt, may be an effective way for the control of wilt disease. Edward and Gaurishanker (1964) in their further studies

found *Psidium cattleianum (Psidium molle), P. quianense*, Chinese guava *(P. friedrichsthalianum)* and Phillippine guava compatible and suggested them for the use of rootstock. A local variety Pei-pa in Taiwan was reported resistant and (*P.friedrichsthalianum*) has been recommended as possible rootstock (Leu and Kao, 1979). Misra *et al.* (2003c) identified F1 population of *Psidium molle* & *Psidium guajava* free from wilt, when grown on wilt sick plot and artificially inoculated repeatedly with *Gliocladium roseum*, *Fusarium solani* and *Fusarium oxysporum*. As graft compatibility is very successful, this resistant rootstock is very useful for the control of wilt.

Disease management through bio-control agent

Due to soil borne nature of wilt pathogen, it is not practical to completely control with any chemical. The effects of chemicals are also hazardous for the soil and environment, moreover when the effect of chemicals diminishes, the pathogen become more virulent and aggressive (Misra and Pandey 1999b). Hence, considering the above facts, it was considered more desirable to use the bio-agents for the management of the wilt disease.

Dwivedi (1992) advocated *Trichoderma spp.* and *Streptomyces chibaensis* for the control of wilt. Seed oil of *Foeniculum vulgare* were also reported to control wilt (Dwivedi, 1993a). In Pakistan, combined use of Topsin M sprays and antagonists and *Arachniotus sp.* added in soil amended with wheat straw controlled decline of guava (Ansar *et al.*, 1994). Logani *et al.*, (2002) reported application of

wiltnema (seven extracts of plants viz., *Allium cepa*, *A. sativum*, *Ocimum sanctum*, *Azadirachata indica*, *Datura stramonium*, *Cannabis sativa* and *Nicotiana tabacum*) for prevention of wilt and better growth of guava plants. Srivastava *et al.*, (2001) found that use of VAM symbiont at the rate of 5kg/tree is beneficial for the control of wilt.

At CISH, Luucknow, three bio- agents were found effective for the control of the wilt disease viz. *Aspergillus niger* strain AN 17, *Trichoderma harzianum*, and *Penicillium citrinum* (Misra *et al.*, 2000, 2004; Prakash *et al.*, 2002). When these fungi were tested for the control of wilt pathogen in laboratory conditions, they were found quite effective (Misra *et al.*, 2004). When relative growth of the three bio-agents was studied, it was found that *Aspergillus niger* was fastest growing and most effective (Misra and Prasad, 2003). These can be grown easily on any substrate like maize/bajra seeds etc. and can also be multiplied on cheap substrates like *Sacchrum sp.* (grass) and dry and green leaves of *Psidium guajava* (Shukla *et al.*, 2003). It was also found that at village level these bioagent can be multiplied in earthen pots (Misra and Prasad, 2004). Among these, *Aspergillus niger* was found very fast growing, easy to propagate and most effective in controlling the wilt disease in field. Besides this quality, it is also growth enhancer and the plants treated with *Aspergillus niger* developed faster with more height, more thickness and more numbers of leaves (Misra *et al.*, 2000). Diwivedi and Shukla (2002)

reported that out of the three bio-agents *Trichoderma harzianum, T. viride* and *Gliocladium virens, T. viride*; was best. (Misra *et al.*, 2003b, d; Misra and Singh, 2005).

Chapter-3
Materials & Methods

MATERIALS AND METHODS

1. Survey for the disease assessment

Periodic survey of guava orchards in adjoining areas of Aligarh district was done during 2008-2009 to determine incidence of guava wilt.

S. No.	Localities in Aligarh district & adjoining areas
1.	Sasni
2.	Chatari
3.	Dhanipur
4.	University farm

In a locality 5-6 fields were selected to evaluate disease incidence and occurrence. The disease incidence and frequency of occurrence was calculated according to the following formula.

$$\textbf{Disease incidence} = \frac{\text{Number of infected plants in an orchard}}{\text{Total number of plants examined}} \times 100$$

$$\textbf{Disease frequency in a locality} = \frac{\text{Number of orchards with infection}}{\text{Total number of orchards surveyed}} \times 100$$

1 a. Isolation of the test organism:

During the survey the infected plant parts stems, roots and rhizosphere soil were collected in polythene bags and brought to the laboratory. The plant parts gently washed with running water to remove adhering dust etc, and then cut into small pieces and surface sterilized with NaOCl (0.05%) for 1-2 minutes followed by 2-3 washings with distilled water. Two to four sterilized pieces were placed aseptically in petriplates containing sterilized and solidified PDA and finally the petri plates were incubated at 25 ± 2^{0}C for 7 days. To obtain pure culture of fungi the superficial growth was subcultured in PDA slants.

Potato Dextrose Agar:

1. Potato (pealed and sliced) - 200 g
2. Dextrose - 20 g
3. Agar - 20 g
4. Distilled water - 1000 ml
5. Streptomycin - 1:3000

1 b. Fungal isolation from rhizosphere:

The rhizosphere soil collected from diseased guava plants were brought to the laboratory in the polythene bags for isolation. One gram of each soil sample was suspended in 9 ml of sterilized water shaken thoroughly to get the soil particles uniformly dispersed in the suspension. 1 ml of suspension from the first dilution (1: 10^{-1}) was aseptically transferred to another tube (10^{-2}) and this procedure

further repeated till the dilution of (10^{-6}) was obtained. For the isolation of rhizospheric fungi 0.1 ml of soil suspension from the dilution of 10^{-4} to 10^{-6} was transferred in sterilized petri plates containing 20 ml Potato dextrose agar medium was taken in tube (10^{-2}) and this procedure further repeated till the dilution was 10^{-6} was the dilution of 10^{-4} to 10^{-6} was transferred in sterilized petriplates containing potato dextrose agar medium, these petriplates were then incubated at 25±2°C for 2-3 days. After incubation the suspected fungal colonies were picked up and used for further studies. Identification of pathogen was made on the basis of their cultural and morphological characteristics.

1 c. Pure culture of the fungus:

Pure culture from single spore was maintained on PDA medium for growth. The culture was maintained at 25±2°C in BOD incubator.

2. Cultural and morphological studies

Visual examination of petri plates was carried out to study cultural characteristics of the fungus. Temporary slides of cultures were prepared in lacto phenol, cotton blue and examined under compound microscope for the mycelium and spore characteristics. The confirmation and specific identification of the culture was done on the basis of morphological characters, shape and size of micro and macro conidia.

3. Effect of different temperature

To study the effect of different temperature on the growth of the pathogenic fungus, Petri plates containing PDA was (20ml/plate) was centrally inoculated with the pathogen fungus (5mm fungal disc) and incubated at temperature 10, 15, 20, 25 and 30°C for 5 days. Three replicates were maintained for each temperature.

4. Use of Biological control agents

Fungal bioagents viz. *Trichoderma harzianum*, *T. virens T. viride* and *Aspergillus niger* were isolated from Faculty of Agricultural Sciences, A.M.U. Aligarh and *Pseudomonas fluorescence* broth was obtained from Department of Microbiology A.M.U. Aligarh.

4 a. In laboratory:

Lab experiments were conducted to evaluate the efficacy of biocontrol agents against pathogenic *Fusarium spp.* In Petri plates by dual inoculation method, 5mm disc of pathogenic fungi (*Fusarium sp.*) and fungal bioagents were placed at 4cm apart in each petriplates containing 20ml of P.D.A. The bacterial bioagent grown on nutrient agar medium where placed in petri plates using 6mm disc of filter on one side of plate and disc of *Fusarium oxysporum* f.sp. *psidii* was placed on the other side and incubated 25±2°C. Observations on the dual culture growth pattern of both the organisms were recorded after

every 24 hrs of inoculation till the fungi/bacteria over grew one another from the inhibition zone.

5. Effect of volatile compounds

Five days old culture of *Trichoderma harzianum* on PDA disc (5mm) was placed aseptically on solidified PDA at the centre of petri plates. The petri plates were incubated at 25±2°C for 2, 4, 6, 8 and 10 days. A set of plates without bioagent at later side *Fusarium oxysporum* f.sp. *psidii* inoculated at upper side served as control. three plates were maintained for each treatment. After incubation the colony diameter of *Fusarium oxysporum* f.sp. *psidii* in different plates was measured and the growth inhibition percent was calculated.

6. Test of plant extracts against pathogen *in vitro*:

Leaf extracts from 10 different plant sp. were collected from different areas of Aligarh to observe their efficacy in control of pathogenic fungus *Fusarium oxysporum* f.sp. *psidii*. About 100 gm of fresh plant leaves were taken, washed thoroughly in distilled water and then crushed in mortar and pestle by adding 100 ml of sterilized distilled water. The crude material was than filtered through double layer muslin cloth and then filtrate was filtered through whatman no. 1 filter paper. The plant extracts so prepared were heated at 40°C for 5 minutes to avoid contamination (Jagnathan and Narsimhan, 1988). Poisoned food technique (Dubey and Patel, 2001) was adopted for evaluation of efficacy of plant extracts *in vitro* against *Fusarium*

oxysporum f. sp. *psidii*.. To examine the inhibitory effect of the mycelial growth, requisite quantities of plant extracts were incorporated into sterile non solidified potato dextrose agar medium (PDA) and shaken well to make it homogenous. The medium was then poured into 100 mm sterile Petri plates @ 20 ml/Petri plate, which were allowed to solidify. Control were maintained without plant extracts in which 5 mm mycelial disc of seven days old culture of pathogenic fungi was inoculated in the centre of Petri plates containing P.D.A.

The observations were taken after every 24 hrs.

Observations:

The radial growth (diameter) of colonies was measured often seven days of inoculation. Per cent inhibition was calculated in comparison to control by following formula as given below (Vincent, 1947).

$$I = C\text{-}T/C \times 100$$

Where, I = Percent inhibition, C= Growth in control, T= Treatment

7. In field condition

7 a. Mass production of the culture of test fungus:

One-kilogram healthy seeds of sorghum were soaked over night in 5% sucrose solution and then such seeds were placed in 500ml Erlynmeyer flask @ 150 gm/flask and autoclaved till it reaches to 15

lbs pressure for 30 minutes, plugged with non-absorbent cotton and covered with butter paper. Pure culture of fungus prepared in the culture tubes was inoculated into these flasks. The inoculated flasks were kept in a B.O.D incubator at the temperature of 27±°C for 7 days. During incubation the flask were shaken thrice daily to ensure proper growth of the fungal mycelium on the sorghum seeds.

7 b. Collection and maintenance of culture of biocontrol agents i.e.:

Fungal biological agents viz. *Trichoderma harzianum*, *T. virens*, *T. viride* and *Aspergillus niger* were isolated from Faculty of Agricultural Sciences, A.M.U. Aligarh and *Pseudomonas fluorescence* broth was obtained from Department of Microbiology A.M.U. Aligarh.

7 c. Mass production of culture of the fungus *Trichoderma harzianum, T. virens, T. viride* and *Aspergillus niger* :

Mass production of culture of *Trichoderma harzianum*, *T. virens* *T. viride* and *Aspergillus niger* is done as described earlier for test fungus in section (7 a.).

7 d. Mass production of *Pseudomonas fluorescence:*

One liter of king's B Broth was prepared and 100 ml was transferred to 250 ml flasks. The flasks were autoclaved at 15 lbs psi pressure for 30 minutes, after being plugged with non absorbent cotton and covered with butter paper.

King's B Broth:-

1. Proteose peptone - 20g
2. Magnesium sulphate ($MgSo_4$) - 6g
3. Potassium sulphate (K_2So_4) - 2.5g
4. Glycerol - 15ml
5. Distilled water - 1 lit

Pure culture of *Pseudomonas fluorescence* was inoculated in the flasks. The inoculated flasks were kept in a B.O.D incubator at a temperature of 35±1°C for 7 days. During incubation the flasks were shaken thrice daily to ensure proper growth of bacterial spore.

7 e. Soil sterilization:

The soil collected from the field of faculty of agricultural sciences A.M.U Aligarh, was used in the present study and steam sterilized in autoclave at 30 lbs psi for one hour. Farm yard manure was also sterilized in the same way.

7 f. Effect of soil treatment with bio-agents on seedling wilt of guava:

Preparation of pots

Pots of 20 cm diameter and 4.5 kg capacity were taken. These pots were filled with sterilized soil and FYM in the ratio of 3:1.

8. Inoculation of fungus and bio-agents in soil

Pots (of 20 cm diameter) containing sterilized soil and FYM were inoculated with *F.o. psidii* @ 5.0 gm / pot. Different treatments comprised of soil application of bio-agents mass cultured on autoclaved sorghum seeds. The bio-agents used were *T. harzianum, T. virens, T.viride, A. niger and Pseudomonas fluorescence.*

8 a. Sowing:

Seedlings of guava were sown in each pot on the fifth day of inoculation. Seedling grown on sterilized soil without any treatment was considered as check.

8 b. Plant growth:

Ninety days after inoculation the plants were carefully uprooted from the pots and their roots were washed under running tap water to remove the adhering soil particles. Excess water was removed with the help of blotting paper. The plant growth was determined by measuring the shoot and root length, fresh and dry weight of roots and shoots. The plant dry weight was determined after drying in oven at 60°C for a sufficient period of time.

8 c. Determination of infection of wilt fungus of *Fusarium oxysporum* f. sp. *psidii*:

The plants were gently uprooted from each pot and the roots were washed through with tap water to remove adhering soil particles. The washed roots of these infected plants were cut separately into pieces of 1.0 cm, then treated with 10 % KOH

solution and finally kept at 90°C FOR 1 hour. These roots segments were washed again with distilled water and then acidified and stained with trypan blue (0.5% in lactophenol) as described by Phillips and Hayman (1970). Twenty to thirty stained pieces were mounted on slides in lactophenol and observed under stereoscopic microscope. The portion of length of root segment which showed the presence of mycelium of the fungus was estimated. The plant infection was calculated by measuring the infected portion in relation to total length of root pieces (Bierrman and Linderman, 1981).

Observations:-

Data regarding the plant growth was recorded 90 days after sowing by measuring the root and shoot heights, fresh and dry weight of roots and shoots.

Observations:

The seedling wilt and percent of wilt control over check was calculated.

Treatments:

The various treatments used to treat the soil were as follows:

T_1 = Control

T_2 = *Fusarium oxysporum* f.sp. *psidii*

T_3 = *T. harzianum*

T_4 = *T. harzianum* + *F.o. psidii*

T_5 = *T. virens*

T_6 = *T. Virens + F. o. psidii*

T_7 = *T.viride*

T_8 = *T.viride + F. o. psidii*

T_9 = *A. niger*

T_{10} = *A. Niger + F. o. psidii*

T_{11} = *Pseudomonas fluorescence*

T_{12} = *Pseudomonas fluorescence + F. o. psidii*

The lengths of shoots and roots were measured. The percentage reduction is obtained by the formula:

Percentage reduction = (T/C × 100) – 100

Statistical Analysis:

The data obtained was subjected to statistical analysis for determining the significance of the various treatments given to the plants treatments given to the plants. The experiments were laid out using completely randomized block design (R.B.D). The data was analysed for least significant difference (L.S.D.) test at the probably of 0.05 to identify significant effect of treatments (Dospecklov. 1984). For the analysis, all the three replicated of treatment were taken into consideration.

Chapter-4
Results & Discussion

RESULTS AND DISCUSSION

Survey were conducted in different localities of district Aligarh to asses the incidence and occurrence of wilt of guava.

Incidence of the disease was assessed in 5 localities of each block viz. Sasni, Chhatari, Dhanipur and university farm were surveyed. Incidence of the disease was highest i.e. 47 % in Chhatari followed by 38 % in Sasni, 32 % in Dhanipur and 21 % in university farm (Table-1).

The frequency of occurrence of the disease was calculated on field surveyed basis, Occurrence of the disease was highest i.e. 82 % in Chhatari followed by 64 % in Sasni, 52 % in Dhanipur and 30 % in university farm.

Isolation and identification of causal organism

Different isolates of *Fusarium oxysporum* f.sp. *psidii* were isolated from infected plant parts of guava from several localities of Aligarh and adjoining orchards of Aligarh district showing the symptoms of wilt. Identification of the fungal pathogen was done on the basis of pathogenecity test and morphological characters. shape and size of conidia etc.that was observed under compound microscope.

Measurement of conidia

The isolates of *Fusarium oxysporum* f. sp. *psidii* possesses variation in cultural characteristics (Plate-2) and in its conidial size

Table 1. Incidence and frequency of occurrence of wilt disease of guava in Aligarh district and adjoining areas.

Locality ---------- %	Frequency of occurrence %	Disease incidence %
Sasni	64	38
Chhatari	82	47
Dhanipur	52	32
University farm	30	21

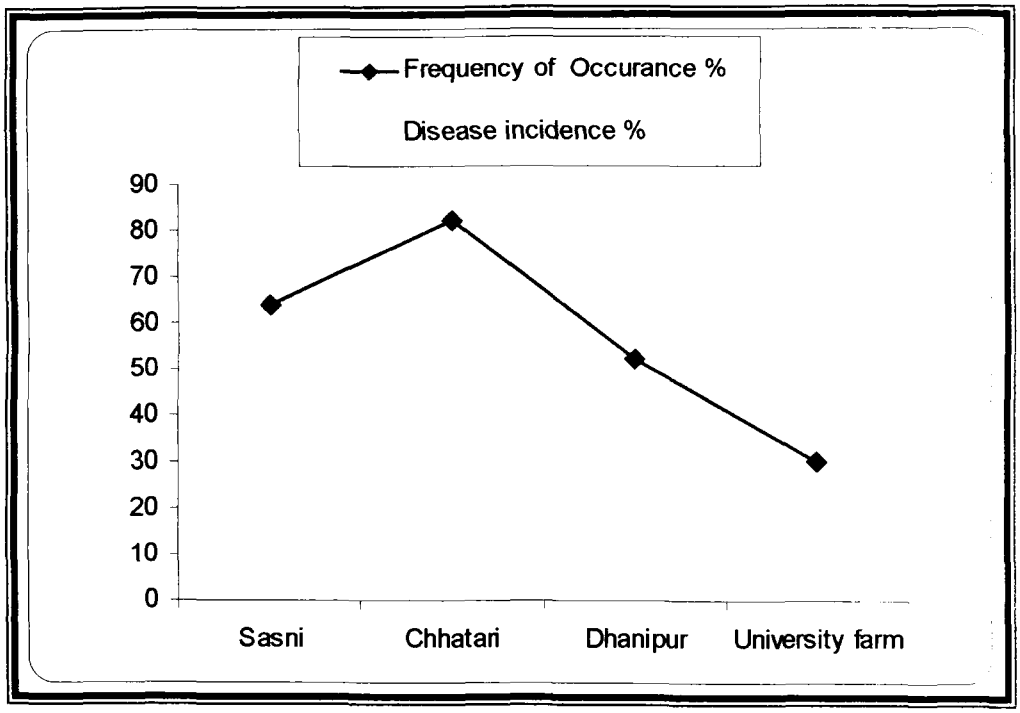


Fig.1.Incidence and frequency of occurrence of wilt disease of guava in Aligarh district and adjoining areas.

and septation (Table-2) The microconidia were found to be oval to ellipsoidal, 1-2 celled macroconidia were found to be elongate curved with the both ends thin, hyaline and three or more septate. The average length of microconidia varied from 6.5-17.5µm. Among all isolates, length of isolate 2 was maximum and minimum size of isolate 1 and its breadth varied from 2.5-6.0 µm. On the other hand, the average length of macroconidia varied from 23-35 µm, being maximum in isolate 2 and minimum in isolate 1 and the breadth varied from 5-6.0 µm. The result is in confirmation with findings of Prasad, Mehta and Lal.

The observations recorded on morphological variations of all four isolates of *Fusarium oxysporum* f. sp. *psidii*, revealed that there were slight differences among the isolates in relation to their conidial length and breadth.

However, slight variations in the conidial size may be attributed to the physical conditions of the medium, yet the variation in all 4 isolates of *Fusarium oxysporum* f. sp. *psidii* in present study visualized glaring differences in conidial size, which gauged on the same nutrient medium (PDA) used for the growth of *F. o.* f. sp. *psidii isolates.*

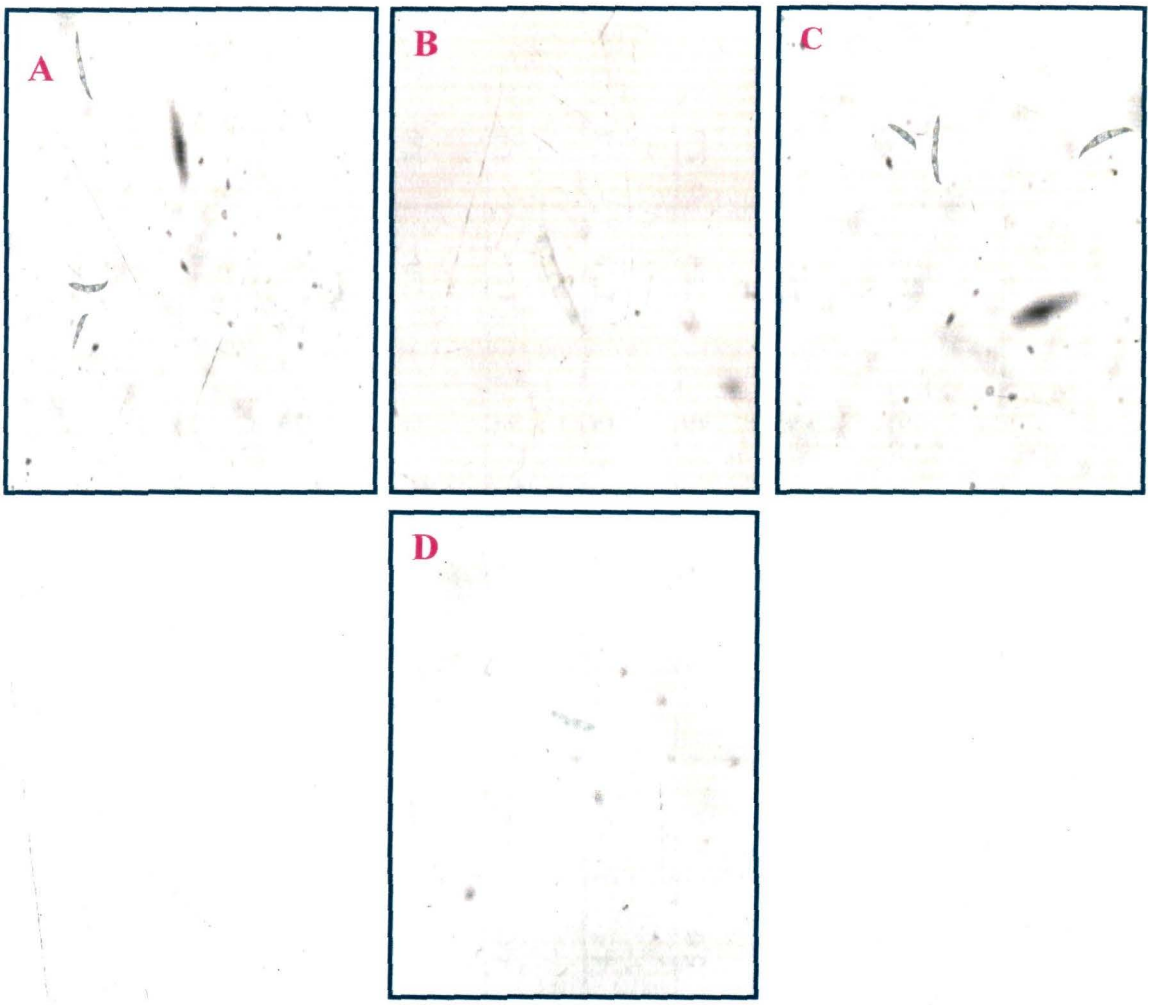


Plate 2- Different isolates of *Fusarium oxysporum* f. sp. *psidii* obtained from adjoining areas of Aligarh, A. Sasni B. Chhatari C. Dhanipur D. University farm

Table 2. Conidial measurement of the Isolate I, Isolate II, Isolate III and isolate IV of Fusarium oxysporum f. sp. psidii.

Micro conidia	Isolate I			Isolate II			Isolate III			Isolate IV		
	Septa	Length (µ m)	Width (µ m)	Septa	Length (µ m)	Width (µ m)	Septa	Length (µ m)	Width (µ m)	Septa	Length (µ m)	Width (µ m)
	0	6.5	3.0	0	8.0	2.5	0	7.5	2.5	0	7.0	3.0
	1	15.0	3.5	1	13.5	4.0	1	12.5	3.5	1	14.0	3.5
Macro conidia	2	17.5	5.0	2	16.0	4.5	2	17.5	5.0	2	17.0	4.5
	3	23.0	5.0	3	25.0	5.0	3	23.5	5.0	3	24.0	5.0
	4	28.0	5.5	4	28.5	6.0	4	30.0	5.0	4	29.0	5.5
	5	32.5	6.0	5	35.5	6.0	5	35.0	5.5	5	34.5	6.0

Effect of different temperature on the growth of the fungus *Fusarium oxysporum* f. sp. *psidii*

As per genetic dictate, evaluation of every organism requires a particular range of temperature, which influence the physiological functioning of the organism, particularly, the enzymatic system

The effect of different temperatures on the growth of fungus was studied. The data recorded is presented in Table (3) and (Fig.2). The data revealed that temperature 25°C is most suitable for maximum growth of fungus followed by 30°C, 20°C, 15°C and 10°C respectively. The growth of the fungus was significantly at a with each other at 30°C and 20°C. The growth at 15°C was significantly different from the growth at 10°C. Schoeman (1996-1998) investigated the effect of temperature on guava wilt disease (caused by *Acremonium diospyri*). He found *in vitro* test 10-35°C and found that the fungus grew best at temp 15-30°C with maximum hyphal growth at 30°C.

Biological management

In vitro evaluation of biological control agents against *Fusarium oxysporum* f. sp. *psidii*

The study was conducted in laboratory using the test fungi *Fusarium oxysporum* f. sp. *Psidii* and biological control agents *T harzianum, T. viridae T. virens, A. niger* and *P. flourescens*. The biological control agents (fungal as well as bacterial) started

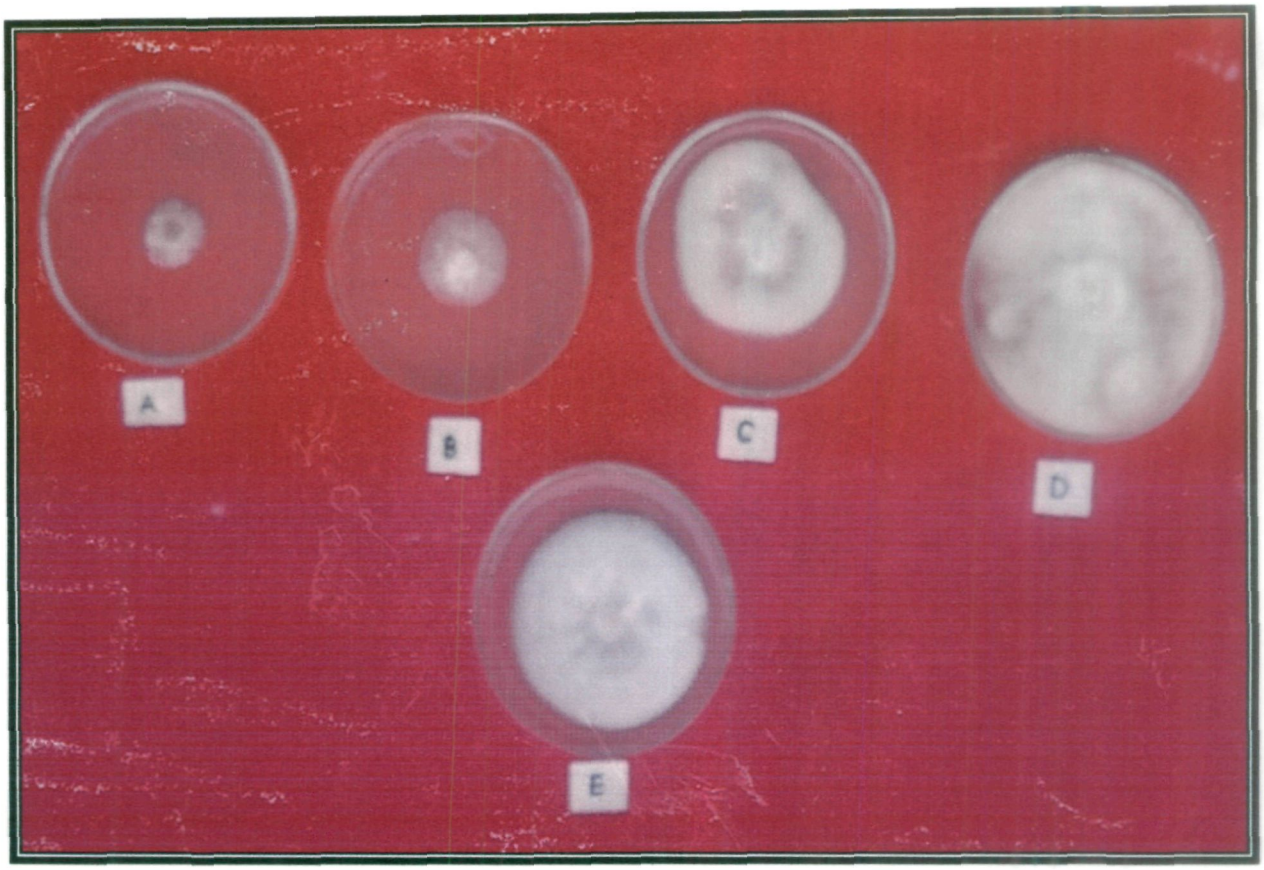


Plate 3- Effect of different temperature on the growth of *Fusarium oxysporum* f. sp. *psidii*.

A- Showing radial growth at temperature 10^0C
B- On temperature 15^0C
C- On temperature 20^0C
D- On temperature 25^0C
E- On temperature 30^0C

Table 3. Effect of different temperature on the growth of *Fusarium oxysporum* f. sp. *psidii.*

Temperature (°C)	Colony diameter (cm)
10	2.0
15	3.1
20	6.0
25	8.5
30	7.1
L.S.D. ($P \leq 0.05$)	0.86

Fig.2. Effect of temperature (^{0}C) on the growth of the *Fusarium oxysporum* f. sp. *psidii*

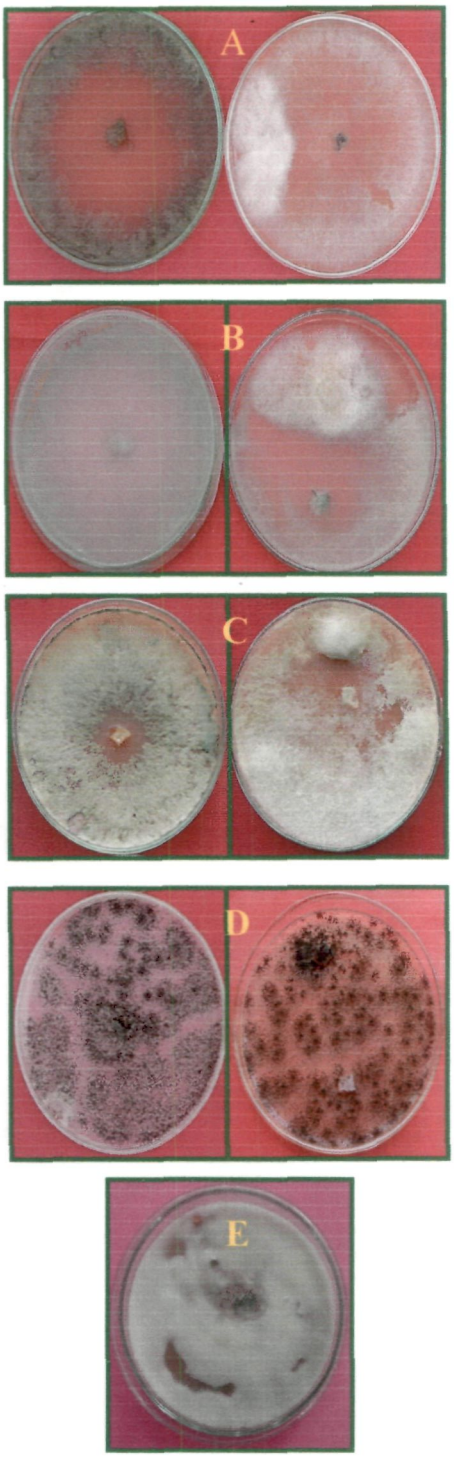
inhibition of growth *Fusarium oxysporum* f. sp. *psidii* after 48 hrs and covered entire petri plate within 6 days (Table 4).

T. harzianum inhibited the growth of *F. oxysporum* f. sp. *psidii* by 44% at 48 hrs. The inhibition of the *F. oxysporum* f.sp. *psidii* increases as *T. hazianum* restricted the growth and inhibition reaches to 69% in 6 days. The results are in confirmation with findings of Misra *et. al.*, and Prakash (2002-04) who applied 3 bioagents in which *T. harzianum* was also used for controlling the wilt disease of guava.

T.viridae was found to be most effective biocontrol agent against *Fusarium oxysporum* f. sp. *psidii*. It reduced the growth by 70% (8.0 cm growth) in 48 hrs. *T. viridae* restricted the growth and inhibition reaches to 75% in 6 days. These results are in accordance with Diwedi and Shukla (2002). They reported that *T. viride* is best for the control of wilt pathogen *Fusarium oxysporum* f. sp. *psidii*.

T. virens was found to be effective against *F. oxysporum* f.sp. *psidii*, *T. virens* completely restricted the growth of the pathogen after 120 hrs. *T. virens* inhibited the growth of *F. oxysporum* f. sp. *psidii* at 48 hrs by 59% (1.1 cm growth). In 6 days 74% inhibition of radial growth was recorded respectively. The results are similar to the findings of Diwedi and Shukla (2002). They reported that *T. virens* was found effective against *Fusarium oxysporum* f. sp. *psidii*.

Aspergillus niger was also found to be effective against *F. oxysporum* f.sp. *psidii*. It reduced the growth by 55% (1.2 cm growth)



A- *Trichoderma virens*, inhibition of *Fusarium oxysporum* f. sp. *psidii* with *T. virens*

B- *T. harzianum*, inhibition of *Fusarium oxysporum* f. sp. *psidii* with *T. harzianum*

C- *T. viride*, inhibition of *Fusarium oxysporum* f. sp. *psidii* with *T. viride*

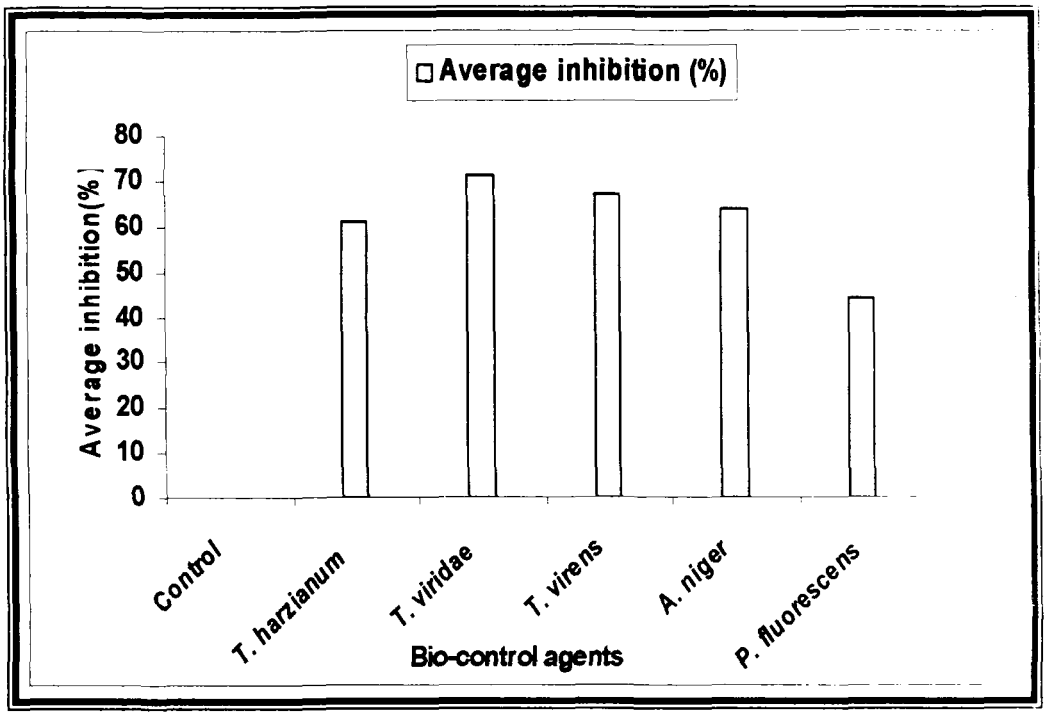
D- *A. niger*, inhibition of *Fusarium oxysporum* f. sp. *psidii* with *A. niger*

E- Control

Plate 4- Dual culture test of *Fusarium oxysporum* f. sp. *psidii* and different bioagents.

Table 4. Effect of biological control agents on the radial growth (cm) of *Fusarium oxysporum* f. sp. *psidii*

Biocontrol agent	48 hours (cm)	72 hours (cm)	96 hours (cm)	120 hours (cm)	144 hours (cm)	Average inhibition (%)
1. Control	2.7	4.5	6.5	8.1	8.5	
2. *T. harzianum*	1.5 (44)	1.8 (60)	2.3 (64)	2.4 (70)	2.6 (69)	61.0
3. *T. viridae*	.8 (70)	1.5 (66)	1.9 (70)	2.1 (74)	2.1 (75)	71.0
4. *T. virens*	1.1 (59)	1.6 (64)	2.0 (69)	2.2 (72)	2.2 (74)	67.0
5. *A. niger*	1.2 (55)	1.9 (57)	2.2 (66)	2.4 (70)	2.4 (71)	63.8
6. *P. fluorescens*	1.8 (33)	2.5 (44)	3.3 (50)	4.2 (46)	4.5 (47)	44.0
L.S.D. ($P \leq 0.05$)	0.33	0.42	0.44	0.11	0.32	



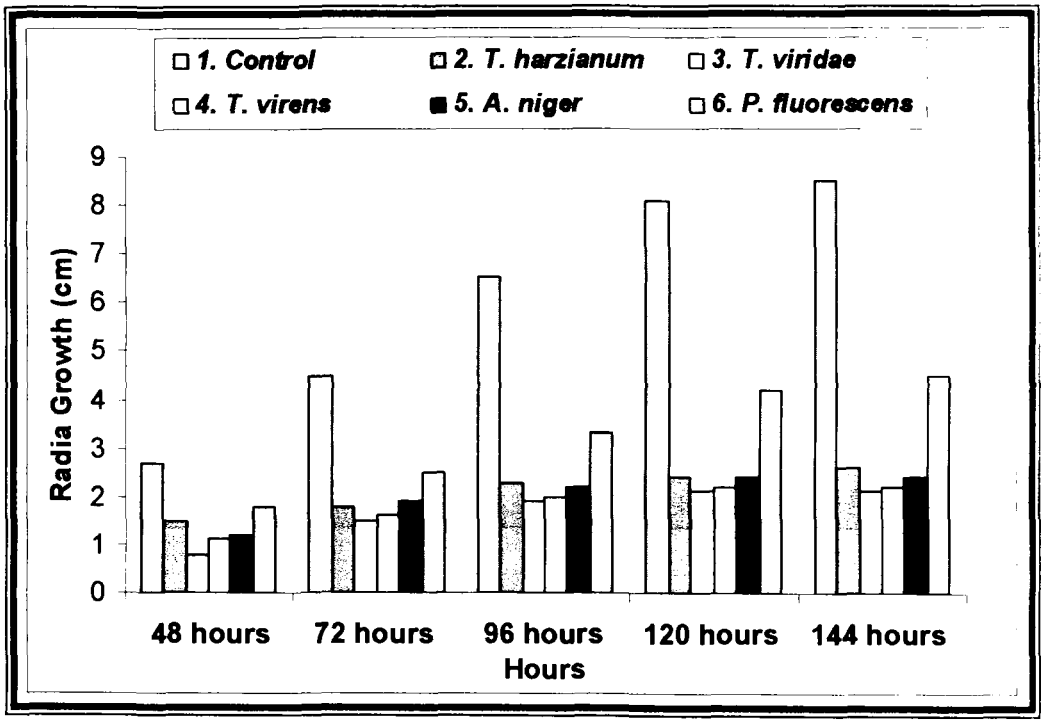


Fig.3. Effect of different biocontrol agents on the radial growth of *Fusarium oxysporum* f. sp. *psidii* at different hours.

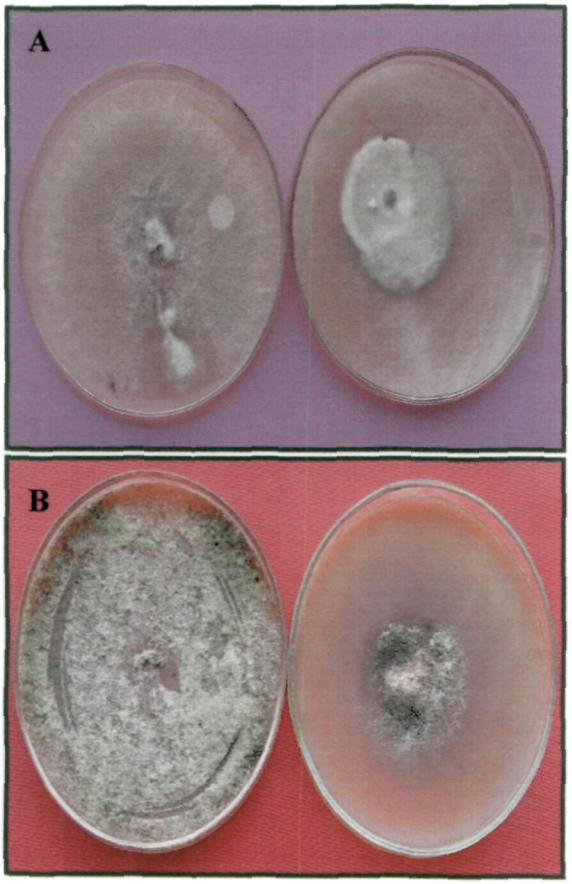
in 48 hrs. *A. niger* restricted the growth of the wilt pathogen and inhibition reaches up to 71% in 6 days. These results are in accordance with the work done by Singh *et.al.* (2003).They reported that the antagonistic fungi *A. niger* is effective against the wilt pathogen *F. oxysporum* f. sp. *psidii*.

Pseudomonas fluorescens bacterial bioagent does not show any remarkable inhibition of the test fungi as compared to other biocontrol agents. At 48 hours inhibition of *F. oxysporum* f.sp. *psidii* was 33% (1.8 cm growth). The growth of the test fungi keep on increasing upto 6 days with a rapid increase between 4 and 6 days. This indicates reduction in inhibition capability of the bioagent i.e. *P. flourescens*.

On observing the average inhibition *T. viridae* was found to be most effective in inhibiting the radial growth ie. by 75% followed by the effect of *T. harzianum*, *T.virens*, *A. niger* and *P. flourescens* respectively.

Effect of volatile compound produced by *Trichoderma harzianum* on the growth *F. oxysporum* f. sp. *psidii in vitro*

The results (Table 5) revealed at 2 days of incubation, volatile compounds produced by *T. harzianum* caused maximum growth inhibition (76.5%) of *F. oxysporum* f. sp. *psidii* followed by 4 days of incubation 63.6% volatile compounds produced by *T. harzianum* at 10 days of incubation caused least inhibition 23.6% on the growth of *F. oxysporum* f. sp. *psidii*. The results are in accordance with the work



A- Control

B- Growth checked by *T. harzianum*

Plate 5- Effect of volatile compounds produced by *T. harzianum* on the growth of *Fusarium oxysporum* sp. *psidii*

Table 5. Effect of volatile compounds produced by *Trichoderma harzianum* on the growth of *Fusarium oxysporum* f. sp. *psidii*.

Periodic interval (days)	Radial growth of *F. oxysporum* f. sp. *psidii* (cm)	Growth (%)
2	2.0	76.5
4	3.1	63.6
6	4.2	50.5
8	5.4	36.4
10	6.5	23.6
Control	8.5	
L.S.D. ($P \leq 0.05$)	0.35	

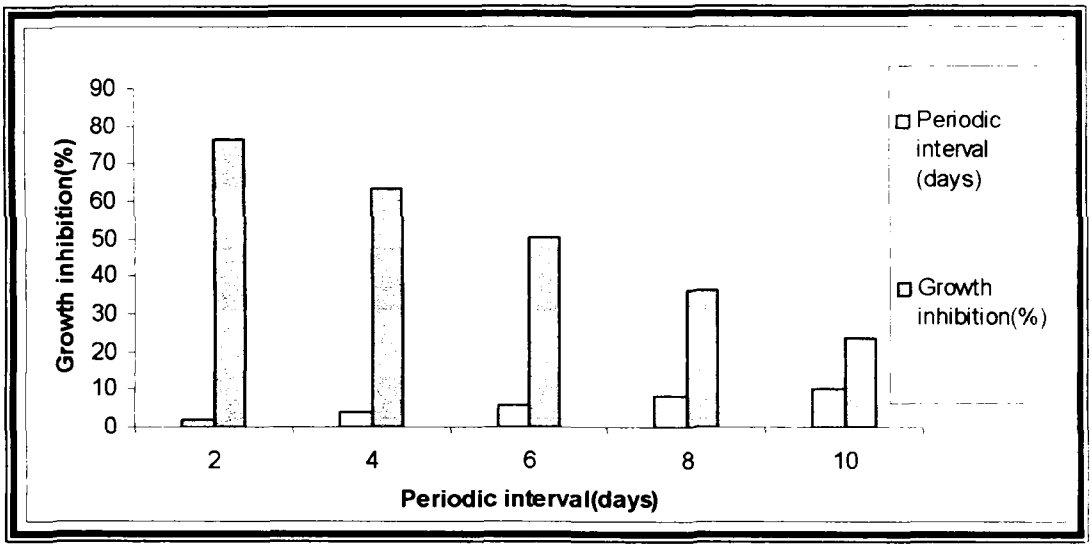


Fig.4. Effect of volatile compounds produced by *Trichoderma harzianum* on the growth of *Fusarium oxysporum* f. sp. *psidii*.

done by Dubey *et al.* (2007) have reported that fresh volatile compounds are better inhibitor of the pathogen fungal growth than older ones.

In vitro evaluation of plant leaf extracts against *Fusarium oxysporum* f. sp. *psidii*

Leaf extracts of all botanicals significantly ($p \leq 0.05$) decreased the radial growth of the pathogen in fungus (table-6). The inhibition of fungal growth was apparently due to the presence of antimicrobial principles of plant extracts which lead to inactivation and death of the pathogen (Taylor, 1984). Plant leaf extracts of *Azadirachta indica* and *Calotropis procera* were found to be highly significant in reducing the radial growth of the pathogen (56.46 % and 51.54% respectively) Grewal and Grewal, (1988) mentioned different fungicidal properties of *Azadirachta indica*, *Chrysanthemum indicum*, and *Tagetes erecta* against various weed moulds of mushroom. leaf extracts of *Parthenium hysterophorus* (46.52%), *Tagets erecta* (43.52), *Solanum nigrum* (41.1%), *Ricinus communis* (39.84%) and *Mentha arvensis* (38.44) showed the same percent inhibition of the growth, the results in accordance with (V.K. gupta et al 2007) who applied different plant leaf extracts against *Fusarium* pathogens of guava (psidium guajava L.). Leaf extracts of *Datura stramonium* (36.92%) and *Nerium indicum* (33.28%) showed the lowest average radial growth inhibition (Reshu, S. Ashraf and F.A. Mohiddin 2007)

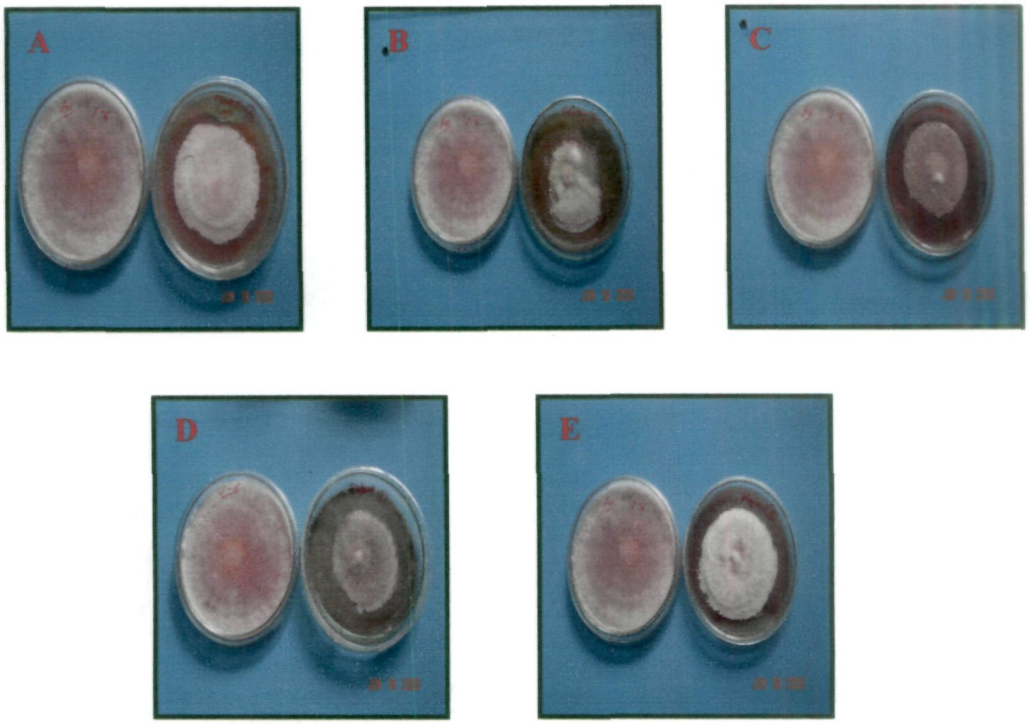


Plate-6 a. Effect of different plant extracts on the redial growth of *Fusarium oxysporum* f. sp. *psidii*. A. *Ricinus communis* B. *Solanum nigrum* C. *Azadirachta indica* D. *Datura stramonium* E. *Nerium indicum*

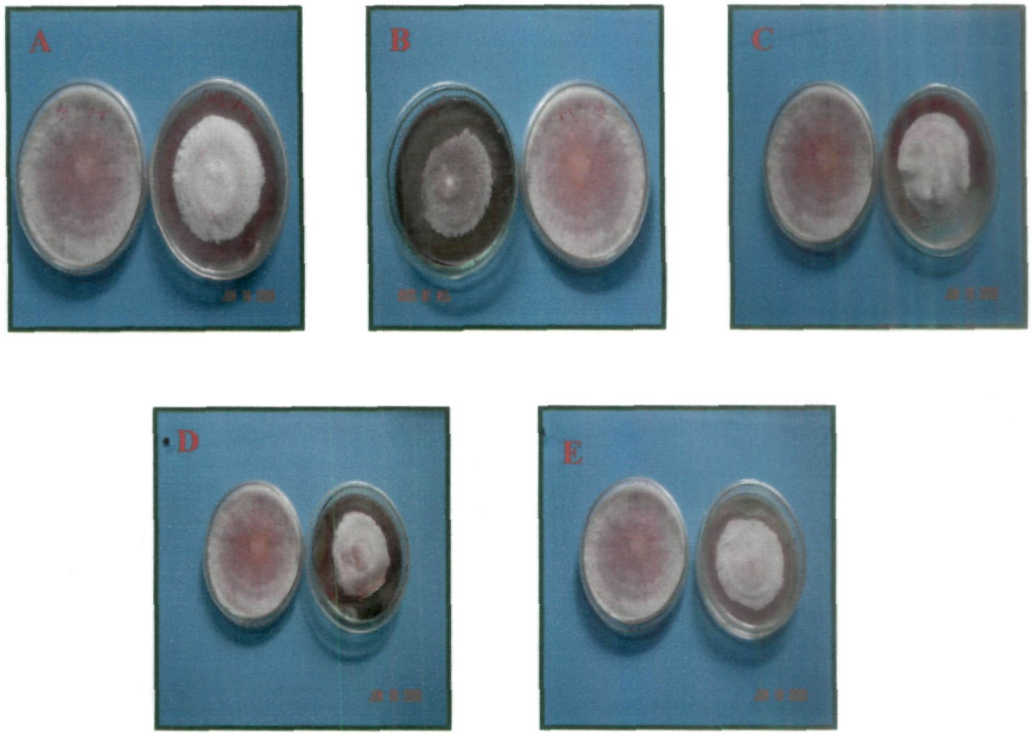
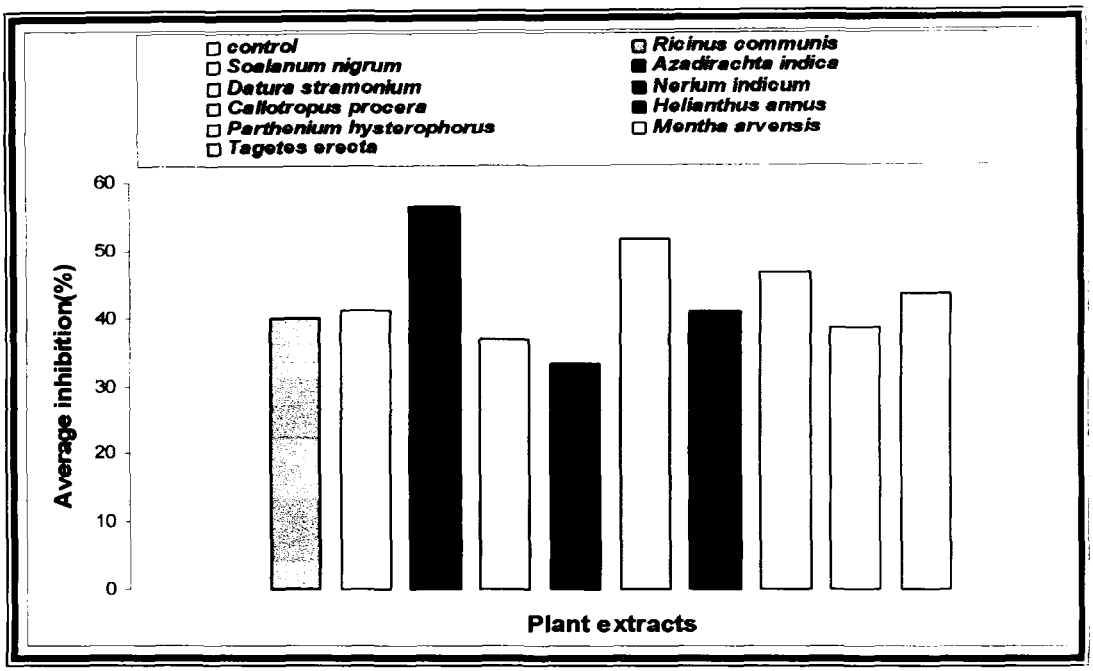


Plate-6 b. Effect of different plant extracts on the redial growth of *Fusarium oxysporum* f. sp. *psidii*. A. *Callotropus procera* B. *Helianthus annus* C. *Parthenium hysterophorus* D. *Mentha arvensis* E. *Tagetes erecta*.

Table 6. Effect of different plant extract on the radial growth of *Fusarium oxyspor um* f. sp. *psidii.*

Plant extract	48 hours (cm)	72 hours (cm)	96 hours (cm)	120 hours (cm)	144 hours (cm)	Average inhibition (%)
Control	2.2	3.6	6.0	7.8	8.5	
Ricinus communis	1.0	1.9	3.4	5.4	6.5	39.84
Soalanum nigrum	0.9	2.0	3.5	5.0	6.4	41.1
Azadirachta indica	0.5	1.4	2.7	3.6	5.5	56.46
Datura stramonium	1.1	2.1	3.8	5.5	6.2	36.92
Nerium indicum	1.5	2.2	3.6	5.0	6.8	33.28
Callotropus procera	0.7	1.5	3.0	4.2	5.5	51.54
Helianthus annus	0.7	1.6	3.9	6.1	6.5	40.76
Parthenium hysterophorus	0.8	1.4	2.8	5.0	6.9	46.52
Mentha arvensis	1.0	2.0	3.2	5.8	6.7	38.44
Tagetes erecta	0.8	1.8	3.0	5.1	6.8	43.52
L.S.D($P \leq 0.05$)	0.29	0.29	0.29	0.29	0.29	



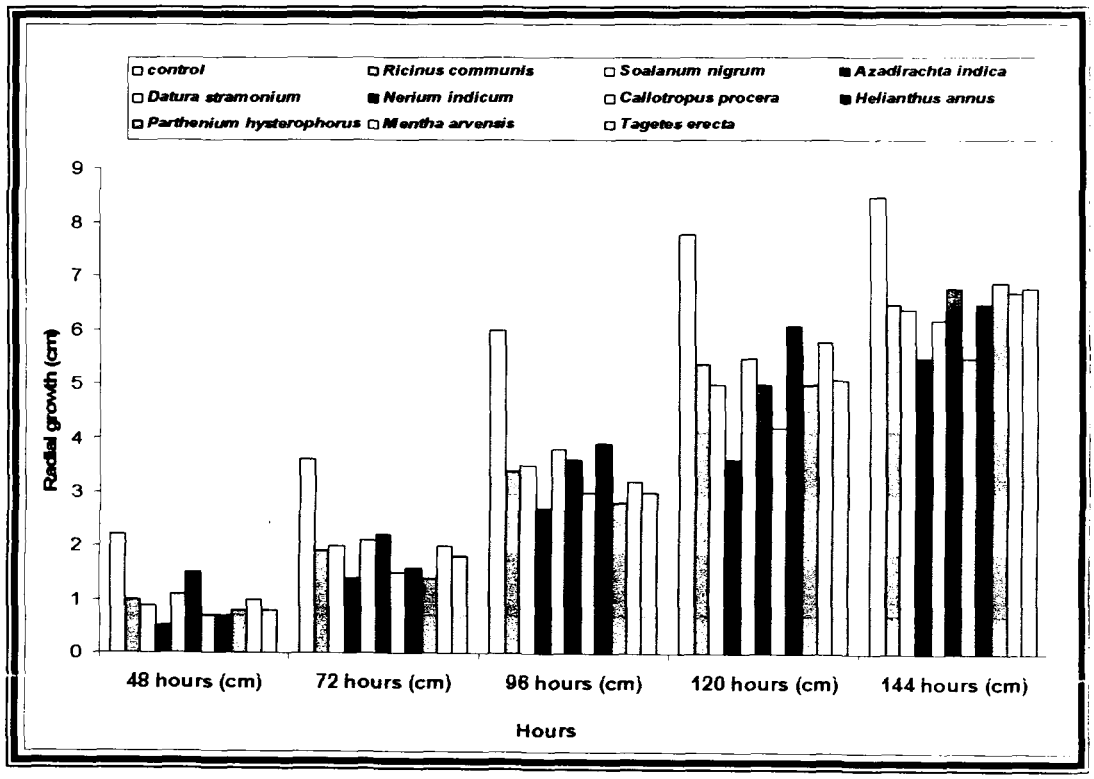


Fig.5. Effect of different plant extract on the radial growth of *Fusarium oxysporium* f. sp. *psidii.*

reported bioefficacy of some botanicals in management of alternaria leaf / blight of Indian mustard (*Brassica juncea* (L.) Czen and Coss).

In vivo evaluation of biological control agents against *Fusarium oxysporum* f. sp. *psidii*

The data presented in Table 7 reveals that the inoculation of guava seedling with fungal pathogen *Fusarium oxysporum* f. sp. *psidii* caused significant reduction in plant growth characters viz. plant length, plant fresh weight, plant dry weight, number of leaves and branches as compared to untreated uninoculated plants. The individual application of fungal biocontrol agent *Pseudomonas flourescens* showed highest increase in shoot length, root length and dry weight of plant was observed in soil treated with *A. niger* in comparison to uninoculated control.

The application of biocontrol agents viz. *T. harzianum, T. viride, T. virens A. niger* and *Pseudomonas flourescens* significantly reduced the damage in terms of plant growth parameters viz. length, fresh weight, dry weight, no. of leaves and branches caused by pathogenic fungus *Fusarium oxysporum* f. sp. *psidii* as compared to untreated and inoculated plants. The highest improvement in growth parameters viz. shoot length (20%), root length (28.5) with *Pseudomonas flourescens* and dry shoot weight (21%), dry root weight (24.5) was recorded in plants treated with, *A. niger* in comparison to uninoculated control respectively after 90 days, where as maximum increase in shoot length (19%), root length

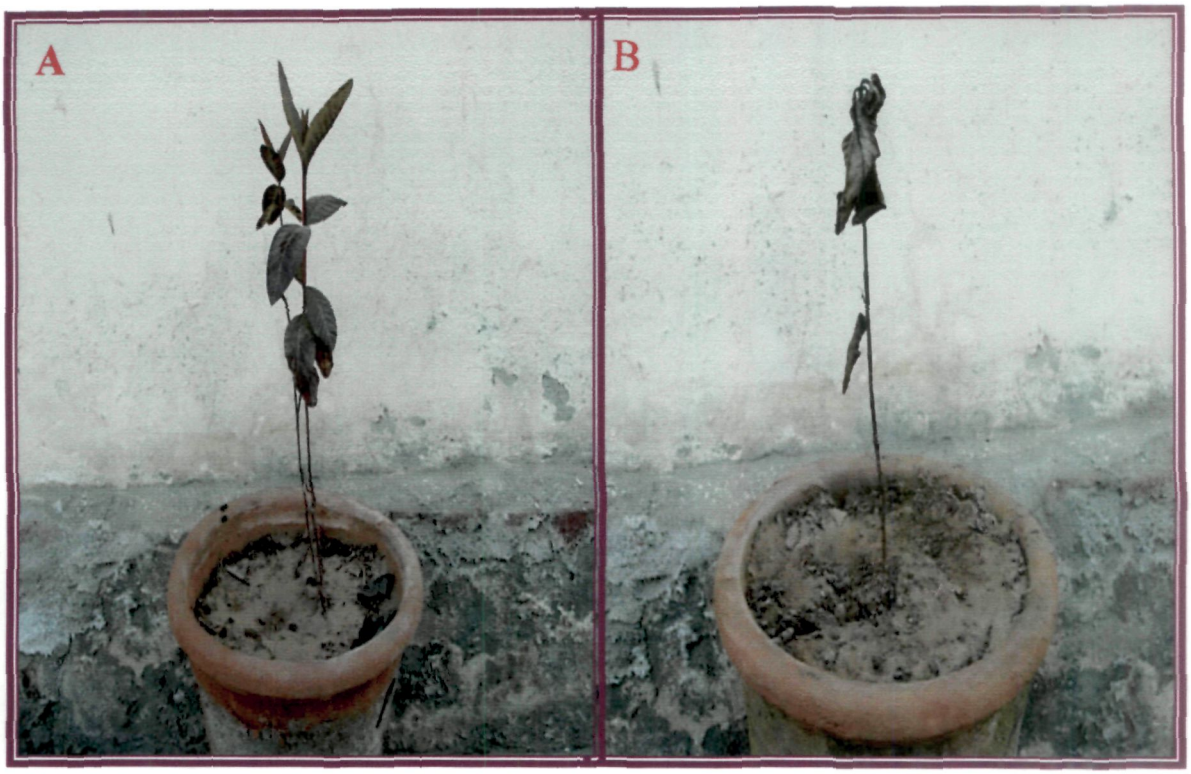
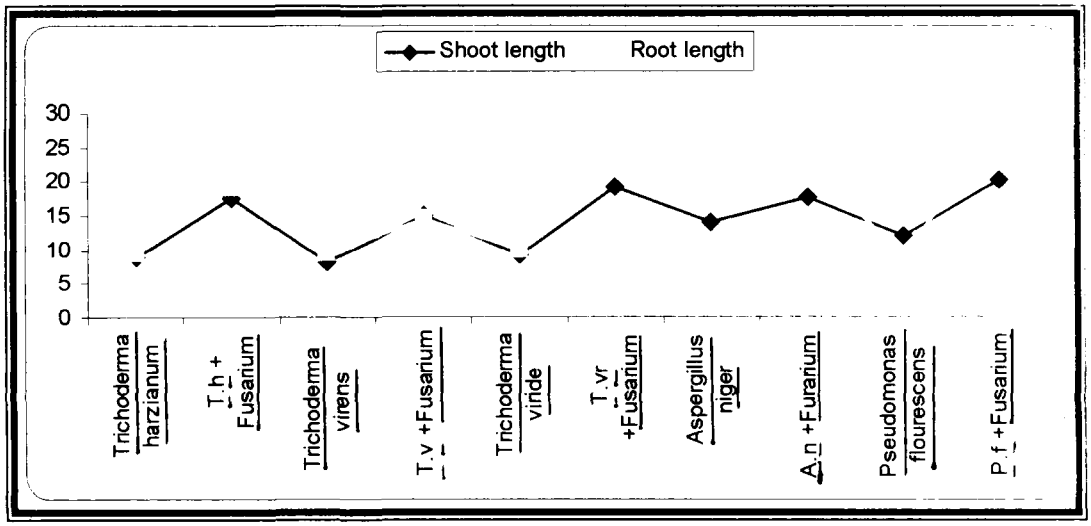


Plate 7- Management of *Fusarium oxysporum* f. sp. *psidii* with bioagents in pots. B. Uninoculated A. Inoculated

Table 7. Management of *Fusarium oxysporum* f. sp. *psidii* with bioagents in pot condition.

Treatment	Shoot length		Root length		Fresh shoot weight		Fresh root weight		Dry shoot weight		Dry root weight	
	(cm)	(%)	(cm)	(%)	(gm)	(%)	(gm)	(%)	(gm)	(%)	(gm)	(%)
Control	22.0		16.0		45		10.2		25.3		5.5	
pathogen	13.5	-38.6	7.5	-53.1	27.6	-38.6	5.5	-46	12.5	-50.9	2.5	-54.5
Trichoderma harzianum	30.1	9.0	24	9.3	60.0	8.0	13.1	7.8	31.5	7.5	7.0	7.0
T.h + Fusarium	16.5	17.5	10.5	19.0	32.6	18	6.5	18	16.6	20.0	3.4	21.0
Trichoderma virens	31.5	8.2	25.4	9.5	61.5	7.2	13.3	8.0	32.5	7.8	7.0	7.5
T.v +Fusarium	17.5	15.0	11.5	14.5	35.3	14.3	7.0	13.5	17.2	13.0	3.6	14.0
Trichoderma viride	34.0	9.2	28	10.0	65.2	08.2	16.5	9.2	36.5	9.0	9.0	10.0
T.vr +Fusarium	18.5	19.0	12.5	25.0	38.6	20.3	8.2	25.0	21.2	22.0	4.3	23.0
Aspergillus niger	31.0	14.0	25.0	23.4	60.9	22.4	13.2	20.0	31.8	21.0	7.2	24.5
A.n +Furarium	17.2	17.5	11.5	23.0	33.6	18.1	6.6	22.0	17.0	7.5	3.3	18.0
Pseudomonas flourescens	25.6	12.0	19.0	9.0	56.2	16.2	12.2	10.0	28.5	11.5	6.1	7.5
P.f +Fusarium	16.2	20	10.0	28.5	28.5	10	7.0	20	14.5	15	3.3	19.0
L.S.D.($P \leq 0.05$)	3.67		2.40		4.97		1.25		2.96		1.74	



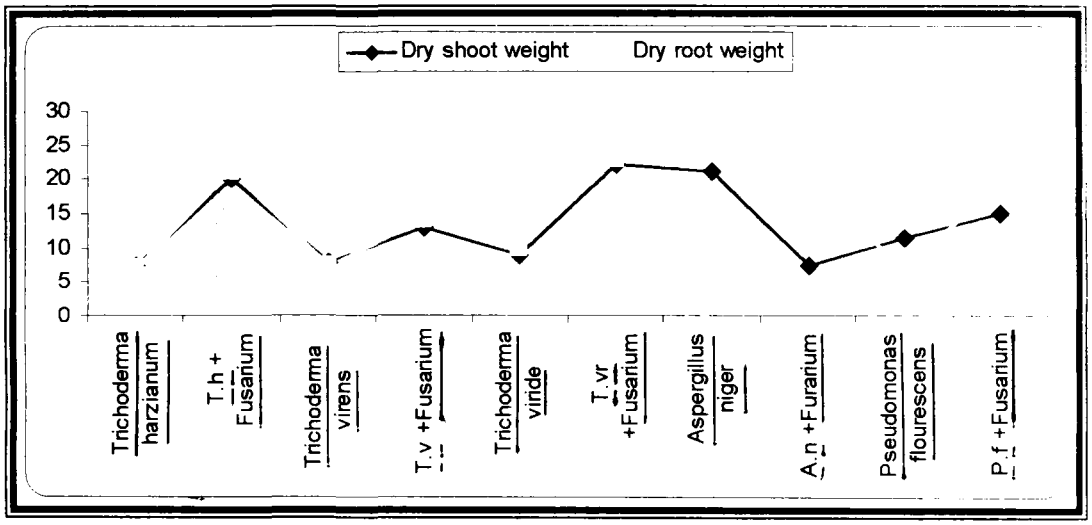


Fig.6. Management of *Fusarium oxysporum* f. sp. *psidii* with bioagents in pot condition.

(25%), dry weight of shoot (22%) and dry weight of root (23%) were recorded with *T. viride* in comparison to inoculated (with *Fusarium oxysporum* f. sp. *psidii*) control pots. The results are in confirmation with findings of Prasad *et al* (2002).

Chapter-5
Summary

Summary

25 Guava orchards in and around Aligarh districts have been surveyed, 82% disease occurrence and upto 47% wilt incidence were observed in Chhatari area of Aligarh. Pathogenic fungus *Fusarium oxysporum* f. sp. *psidii* were isolated from diseased plants as well as from rhizospheric soil to know the variation. *In vitro* experiments were conducted to study the size of conidia, and effect of temperature on growth of the pathogen. *In vitro* antagonistic effects of fungal and bacterial biofungicides and with plant extracts and *in vivo* management of the disease with the help of fungal and bacterial biological agents. Among all tested isolates the average size of micro conidia were found to be 10.75 X 3.25 µm ranged with 15 X 4 µm to 6.5 X 2.5 µm. The average size of macro conidia were found to be 26.5 X 5.5 µm ranged with 35.5 X 6 µm to 17.5 X 5 µm.The maximum radial growth of *F. oxysporum f. sp. psidii* was found at temperature 25°C with visible growth between 8-47°C. Among all bioagents (*Trichoderma harzianum, T. virens, T. viride A. niger* and *Pseudomonas flourescens*) used against *F. oxysporum* f. sp. *psidii* in dual inoculation test, the maximum growth inhibition was recorded with *T. viride* (75%). Effects of volatile compounds produced by *T harzianum* were seen on the growth of the pathogen and found that 2 days of incubation with *T. harzianum* gave a maximum growth inhibition of 76.5% followed by 4, 6, 8 and 10 days of incubation with 63.6 %, 50.0% 36.4%, 23.6% of inhibition, respectively and it

was found that fresh volatile compounds were more inhibitory effects for the growth of the pathogen fungus *(in vitro)* than the later ones. Among ten plant extracts of (*Ricinus communis, Soalanum nigrum, Azadirachta indica, Datura stramonium, Nerium indicum, Callotropus procera, Helianthus annus, Parthenium hysterophorus, Mentha arvensis, Tagetes erecta*). The maximum i.e. 56.46% growth inhibition of *F. oxysporum f. sp. psidii* was recorded with *Azadirachta indica* on PDA. Highest increase in shoot length (20%) and root length (28.5%) were recorded with *P. flourescens* and dry weight of shoot (21%) and root (24.5%) with *A. niger* in comparison to uninoculated control pots, were as maximum increase in shoot length (19%), root length (25%), dry weight of shoot (22%) and dry weight of root (23%) were recorded with *T. viride* in comparison to inoculated (with *F. oxysporum f. sp. psidii*) control pots.

Chapter-6
References

REFERENCES

Agrawal, D.K. and Sarbhoy, A. B. (1978). Physiological studies of four species of Fusarium pathogenic to soybean. *Indian Phytopath.*: 24-31.

Anonymous (1949). Principal administrative report of the Department of Agriculture, united provinces for the year 1947-48. pp. 89.

Anonymous (1953). Report: Directorate of plant protection. Quarantine and storage (1946-1951). Govt. of India.

Anonymous, (1999). NHB, Database National Horticulture Board Gurgaon. India. pp. 43-45.

Anonynous (1996). DBT-GOI project on biological control approaches in the management of *Parthenium hysterophorus* L., *Solanum elaeagnifolium* and *Eupatorium glandulosum.* Annual report 1994-1996. Deptt. Of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India.

Ansar, M.; Saleem, A. and Iqbal, A. (1994). Cause and control of guava decline in Punjab. *Pakistan J. Phytopathological.* 6: 41-44.

Bhargava, A.K., Sobti, A.K. and Ghasalia, R.P. (2003). Studies on guava (*Psidium guajava* L.) drying/wilt disease in orchards of Pushkar valley. *J. Phytopathological Research.* 16: 81-84.

Chakraborty, D.K. and Singh, R.N. (1989). Guava correlation between variations in disease syndrome and edaphic factors. *Indian J. Phytopath.*, 42: 310.

Chandra Mohan, (1985). Studies on guava decline in Punjab Agricultural University. Ludhiana.

Chattopadhya, S.B. and Battacharya, S.K. (1968). Investigation on wilt disease of guava (*Psidium guajava* L.) in West Bengal. *Indian J. Agri. Sci.*; 65-72.

Chattopadhya, S.B. and Sengupta, S.K. (1955). Studies on wilt of *Psidium guajava* L. in West Bengal. *Indian J. Hort.*, 12: 76-79.

Das gupta, M.K and Ghoshal, B.K. (1977). Is it possible to control guava wilt through oil cake amendments? *Sci. and Cult.* 43: 131-133.

Das Gupta, S.N. and Rai, J.N. (1947). Wilt disease of guava. (*Psidium guajava* L.) *Curr. Sci.*; 16: 257-258.

Dubey, S.C.; Suresh, M and Birendra Singh (2007). Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceri* for integrated management of chickpea wilt. *Biological Control* 40: 118-127.

Dey, P.K. (1948). *Plant Pathology*. Annual Administrative report. Deptt. Of Agri., United Provinces (1946-47). pp. 43-46.

Dhingra, O.D. and Sinclair, J.B. (1986). *Basic Plant Pathology methods*. CRC Press Inc. Boca Raton, Florida, pp. 280-339.

Dwivedi, B.P. and Shukla, D.N. (2002). Biocontrol of Fusarium wilt of Guava (*Psidium guajava* L.) using *Trichoderma* and *Gliocladium* species. *Karnataka J. Agri. Sci.* 15: 399-400.

Dwivedi, S.K. (1992). Effect of culture filtrate of soil microbes of pathogens incitig wilt disease of guava (*Psidium guajava* L.) under *in vitro* conditions. *Natl. Acad. Sci. Letter* 16: 7-8.

Dwivedi, S.K. (1993b). Soil solarization adversely affects some fungal pathogens causing wilt disease of guava. (*Psidium guajava* L.). *Soil Bio. Biochem.*, 25:11.

Dwivedi, S.K. (1990). Guava wilt incited by *Macrophomina phaseolina*. *National Academy Sci., Letter.* 13:301-303.

Dwivedi, S.K.; Misra, R. and Dwivedi, R.S. (1990). Incidence of wilt disease of guava (*Psidium guajava* L.) in Varanasi. *Int. J. Trop. Plant Disease.* 6: 213-316.

Edward, J.C. and Gaurishanker (1964). Root stock trial for guava (*Psidium guajava* L.). *The Allahabad Farmer*, 38: 249-250.

Edward, J.C. (1960). Wilt of guava. *Allahabad Farmer.* 34: 289-293.

Edward, J.C. (1961). Root stock trials for guava wilt control. *The Allahabad Farmer*, 35: 5-9.

Edward, J.C. and Srivatava, Q. N. (1957). Studies on guava wilt. *Allahabad Farmer.* 31 : 144-146.

Grech, N.M. (1985). First report on Guava rapid death syndrome caused by *Septifusidium* sp. in South Africa. *Plant Disease*, 69(8) : 726.

Gupta, P.; Dubey, V.; David, A. and John, D. (2003). *Verticillium albo-atrum* – a new pathogen associated with guava wilt. *Indian Phytopath.* 56: 503-504.

Hamiduzzaman, M.M.; Meat, M.B. and Ahmad, M.U. (1997). Effect of *Fusarium oxysporum* and nematode interaction on guava wilt. *Bangladesh J. Pl. Pathol.* 13: 9-11.

Hsieh, S.P.Y. Liang, W.J.; Kao, C.W. and Lell, L.S. (1976). *Plant Prot. Bull.*, (Taiwan), 18: 309-17.

Jain, S.S. (1956). A preliminary not on the inactivation of *Fusarium oxysporum* f. psidii in guava plants by chemotherapeutic treatment. *Indian J. Hort.* 13: 102-104.

Jain, S.S. (1956). A preliminary note on the inactivation of *Fusarium oxysporum* f. *psidii* in guava plants by chemotherapeutic treatment. *Indian J. Hort.*, 13: 102-104.

Jeyalakshmi, C.; Doraiswamy, S.; Valluvaparidasan, V. and Rabindran, R. (2001). Effect of substrate media on virulence of *Fusarium pallidoroseum*: a potential bio-herbicide for parthenium management. *Indian Phytopath.* 54 (4): 453-56

Jhamaria, S.L. (1972). Nutritional requirements of *Fusarium oxysporum* f. sp. *niveum*. *Indian Phytopath.*, 25: 29-32.

Jhooty, J.S.; Chand, J.N. and Krishnamurthy, V. (1984). Report. Guava decline in Punjab and Haryana. In: consolidated report of multi-location project on guava decline. pp. 34-41.

John, T. (1969). Plant pathological methods. Fungi and bacteria. Burgress Publishing company, pp. 239.

Joubert, M.H. and Frean , R. T. (1993-94). An *in vitro* evaluation of fungicides aginst guava wilt. *Inligtings Bulletin Instituut Vir Tropiese en Subtropiese Gewasse*. No. 246: 3.

Joubert, M.H. and Frean, R.T. (1993). An in-vitro evaluation of fungicides against guava wilt. *Inligtings Bulletin Instituut Vir Tropiese en Subtropiese Gewasse*. No. 246: 3.

Junqueira, N.T.V.; Andrade, L.R.M.; de, Pereira M.; Lima M.M. and Chaves, R.da.C. (2001). Diseases of guava (*Psidium guajava* L.) cultivated in Brazilian Cerrdos. Circular Technica Embrapa Cerrados 15: 31.

Kakkar, R.K. and Mehrotra, B.R. (1972). Studies on imperfect fungi II. The activities of hydrogen and hydroxyl ions. *Sydowia* 25: 89-99

Khan, R.M and Misra, A.K. (2003). Infuence of co-cultivation of marigold, garlic and turmeric on nemic population in guava cropping system. Souvenir & Abstract, Zonal Conference (East Zone), Indian Society of Mycology and Plant Pathology & Seminar on Plant Diseases of National Importance with Special

Reference to Guava Wilt and Mango Malformation. 4-5 th April. 2003. CISH and IISR. Lucknow. Pp. 24-25.

Khan, R.M. ; Kumar, S. and Parvath Reddy, P. (2001). Role of plant parasitic nematode (s) and fungi in guava wilt. *Pest Management in Horticulture Ecosystem* 7: 152-161.

Leu, L.S. and Kao, C.W. (1979). Artificial inoculation of guava with Myxosporium psidii. Plant Dis. Reptr.63: 107.

Lim, T.K. and Manicom, B.Q.(2003). Diseases of guava. In: *Diseases of tropical fruit crops*. Pp. 275=289. (Ed. R.C. Ploetz) Pub. CABI, Wallingford, U.K.

Logani, R.; Pandey R.C.; Dwivedi B.K. and Singh S.P. (2002). An ecofriendly management of wilt disease of guava by wiltnema (a botanical fungicide). *Bioved* 13: 47-50.

Mathur, R.S., (1956). Guava diseases in India. *Indian J. Hort.*, 13:26-29.

Mathur, R.S.; Jain S.A. and Swarup J. (1964). Chemical treatment for guava wilt. *Proc., Nat. Acad. Sci.*, 34: 33-36.

Meena, B. Ramamurthy, V.; Marimuthu and Velaznahan, R. (2000). Pseudomonas flourescens mediated systemic resistance against late leaf spot of groundnut, *Journal of plant Pathology. Tamil Nadu Agricultural University, Coimbatore* 641003. Tamil Nadu, India.

Mehta, N. (1987). Distribution of guava wilt in relation to age, soil

type, management practices and varieties grown in Haryana. *Plant Diease Res.* 2 : 116-119.

Mehta, N. (1987). Distribution of guava wilt in relation to age, soil type, management practices and varieties grown in Haryana. *Plant Dis. Res.* 2:116-119.

Mehta, P.R. (1951). Observations on new and known diseases of crop plants in Uttar Pradesh. *Plant Prot. Bull.*, 3: 712.

Misra, A.K. (2003). Guava diseases – their symptoms, cause and Management. In: *Diseases of fruits and vegetables: Diagnosis and management.* Vol. 2 (ed. S.A.M.H. Naqvi). Pub. Kluwer Academic Publishers, The Netherlands. pp. 81-119

Misra, A.K. (2005). Important diseases of guava in India with special reference to wilt. Souvenir. *1st International Guava Symposium.* December 5-8, 2005. CISH, Lucknow. pp.15-90.

Misra A.K. (2006) Wilt of guava – a disease of national importance. *Indian phytopath.* 59 (3): 269-280.

Misra, A.K. and Pandey, B.K (1999b). Pathogenicity and evaluation of fungicides against guava wilt pathogens. *J. Mycol. Pl. Pathol.* 29: 274-275.

Misra, A.K. and Pandey, B.K. (1992). Wilt of guava and associated pathogens. *Indian J. Mycol. Pl. Pathol. 22: 85-86.*

Misra, A.K. and Pandey, B.K. (1994-1995). Studies on guava wilt. Annual Report. Central Institute for subtropical Horticulture. Lucknow. pp. 28.

Misra, A.K. and Pandey, B.K. (1997). Pathogenicity and symptom production of wilt disease of guava by a new potent pathogen. *Gliocladium roseum*. International conference on Integrated Plant disease management for sustainable Agriculture. IARI, New Delhi. pp.28.

Misra, A.K. and Pandey, B.K. (2000b). Progressive natural wilting of guava plants during different months. *Indian Phytopathol*. 53: 423-427.

Misra, A.K. and Pandey, B.K. (1999a). Natural wilting of guava plants during different months. 51 st Annual Meeting and Nat. Symp. On Seed health care and Phytosanitation for Sustainable Agriculture.17-19 Feb. 1999, IISR, Lucknow. pp. 64-65.

Misra, A.K. and Pandey, B.K. (2000a). Pathogenicity and symptom production of wilt disease of guava by a new potent pathogen *Gliocladium roseum*. Proceedings, Indian Phytopathological Society Golden Jubilee, International Conference on Integrated Disease Management for Sustainable Agriculture Vol. II Pub. *Indian Phytopathological Society*, New Delhi, pp.149-150.

Misra, A.K. and Prakash, Om (1986). Studies on diseases of fruit crops. Annual Report. CINHP, Lucknow.pp. 67-68.

Misra, A.K. and Prasad, B. (2003). Relative efficacy of different bio-agents for the control of guava wilt. *J. Mycol. Pl. Pathol*. 33: 494.

Misra, A.K. and Prasad, D. (2004). *Aspergillus niger* strain AN 17

potent bioagent to control wilt disease and its easy multiplication. Symposium on recent advances in fungal bioagents and their social benefits, 10th Sept. 2004, NBRI. Lucknow. pp. 12.

Misra, A.K. and Shukla, S.K. (2002). Assessment of loss due to Guava wilt around Lucknow. Nat. Seminar on Production and Post Harvest Technology of Guava. Dept. Hort. CSAUA&T.Kanpur, 9-10 January, 2002, pp. 34-35.

Misra, A.K. and Singh, V.K. (2005). The CISH, Lucknow, marches towards. Solving guava wilt and mango malformation. *Indian Horticulture* (April-June), 50(1): 25-27.

Misra, A.K.; Prasad, D.; Prasad, B. and Shukla, S.K. (2004b). Effective management of wilt disease of guava. Souvenir & Abstract. Natn. Symp. On Crop Society. Feb. 19-21. 2004. Div. of Plant Pathology, IARI, New Delhi, pp. 92-93.

Misra, A.K.; Om Prakash and Sen, B. (2000). Biological control of guava wilt by *Aspergillus niger* strain AN17 (Pusa Mrida). Nat. Seminar on Hi-tech Hort. 26-28th June, Banglore, pp. 149.

Negi, S.S.; Misra A.K. and Rajan, S. (2001). Guava wilt. Proc. National Seminar on New Horizon in Production and Post Harvest Management of Tropical and Subtropical Fruits, IARI New Delhi, Held on Dec. 8-9, 1998, Special issue, Indian Journal of Horticulture, 54(1&2), March & June: 145-151.

Pal, B.P. (2002). Wilt resistant guava a possibility. Pantnagar News.

5(3):4.

Pandey, R.R. and Dwivedi, R.S. (1985). *Fusarium oxysporum* f. sp. *psidii* as a pathogen causing wilt of guava in Varanasi district of India. *Phytopath. J.* ; 114: 243-248.

Pandey, R.R.; Arora, D.K. and R.C. Dubey (1993). Antagonistic interaction between fungal pathogens and phylloplane fungi of guava. *Mycopathologia.* 124: 31-39.

Pandit, P.K. and Samajpati, N. (2002). Wilt disease of guava (*Psidium guajava* L.) by Botryodiplodia theobromae *Pat. J. Mycological Res.* 40: 71-73.

Prasad, D.; Shukla S.K.; Babita Prasad and Misra, A.K. (2003). Effect of intercrop and different doses of NPK on the incidence of guava wilt. Souvenir & Abstract, Zonal Conference (East Zone), Indian Society of Mycology and Plant Pathology & Seminar on Plant Diseases of National Importance with Special Reference to Guava Wilt and Mango Malformation. 4-5 th April, 2003. CISH and IISR.Lucknow, pp. 53.

Prasad, N.; Mehta, P and Lal, S.S. (1952). Fusarium wilt in Uttar Pradesh. India. *Nature.* 169:753.

Rodrigues, N.J.; Robbs C.F. and Yamashimo T. (1987). A bacterial disease of guava (*Psidium guajava* L.) caused by *Erwinia psidii* sp. *Fitopathologia Brasileira.* 12: 345-350.

Rodriguez, F.M.E. and Landa, J.B. (1977). Chemical soil

disinfection against parasitic nematode in guava nurseries. Centrol Agricola de la Facultod de Cieneias Agricolos, 4: 57-77.

Schoeman, M.H. (1996-1998). Effect of temperature on guava disease development. 276, 5-6.

Sen, P.K and Verma , B.S. (1954). Studies on the die back disease of guava (*Psidium guajava*) – A survey of the incidence of the disease in Jhargram area (West Bengal). *Proc. of the 41st Indian Sci. Cong., Sec. X. Agric. Sci.* pp 258.

Shikhamany, S. D. and Sudha, M. (2004). Lucrative export possibility. *The Hindu survey of Indian Agriculture*, 122-125.

Shukla, S.K.; Babita Prasad, Prasad, D. and Misra A.K. (2003). A cheap substrate for the mass multiplication of bio agents of guava wilt. Souvenir & Abstract, Zonal Conference (East Zone). Indian Society of Mycology and Plant Pathology & Seminar on Plant Diseases of National Importance with Special Reference to Guava wilt and Mango Malformation. 4-5 th April, 2003. CISH and IISR, Lucknow, pp. 54-55.

Singh, B. and Lal, S.B. (1953). Wilt of guava. *Agri. and Animal Husbandry* III: 78-79.

Singh, G. and Reddy, Y.T.N. (1997). Regulation of cropping in guava. *Indian J. of Horti.* 54(1): 44-49.

Singh, G. and Roa, G.P.S. (1996). Regulation of cropping in guava. *Indian J. of Horti.* 53(2): 125-128.

Singh, S.H.; Ved Ratan; Gaur G.S. and Gangwar, D. (2003). Evaluation of antagonists against guava wilt pathogen. *Farm Science J.* 12: 58-59.

Srivastava, A.K.; Ahmad, R.; Kumar S. and Sukhada Mohandos (2001). Role of VA- Mycorrhiza in the management of wilt disease of guava in the alfivols of Chotanagpur. *Indian Phytopath.* 54: 78-81.

Suhag, L.S. and Khera, A.P. (1986). Studies on the variation in nutritional level of wilted, regenerated and healthy trees of guava cultivar Baranasi Surkha *Indian Phytopath.*, 39: 90-92.

Suhag, L.S. (1976). Observations in guava decline in Haryana and its control. Pesticides 10: 42-44.

Tendon, R.N. and Agarwala, R.K. (1954). Pathological studies of *Gleosporium psidii* causing die back of guavas. *Proc., Indian Acad. Sci.*, 40(4): 102-109.

Tokeshi, N.; Valdebenito R.M. and Dias, A.S. (1980). Occurrence of a bacterial disease of guava in Sao Paulo state. *Summa Phytopathologica*, 6: 85-87.

Vestal, E.F. (1941). Text Book of Plant Pathology Kitabistan, Allahabad. pp. 406.

Vos, J.E.; Schoeman, M.H.; Berjak, P.; Watt, M.P.and Toerien, A.J. (2000). *Acta Horticultural* 513: 69-79.